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List of abbreviations

AMF: Arbuscular Mycorrhizal Fungi

ANOVA: Analysis of variance

DM: Dry Matter

GLM: Generalized Linear Model

GSL: Glucosinolate

IC: Intercropping

ITC: Isothiocyanates

LER: Land Equivalent Ratio

MeBr: Methyl Bromid

MIX: Mixture

MSCC: Multi-Services Cover Crops

SC: Sole Crop

Aknowledgements

Whoa! I am crossing the finish line of this PhD space trip. It was a pleasure and a great adventure mainly thanks to all my travel mates that I want to warmly thank.

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List of publications coming from this PhD

International journals:

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Couëdel, A., Alletto, L., Kirkegaard, J., Justes, É., 2018. Crucifer glucosinolate production in legume-crucifer cover crop mixtures. Eur. J. Agron. 96, 22–33. doi:10.1016/j.eja.2018.02.007

Couëdel, A., Alletto, L., Justes, É., 2018. Crucifer-legume cover crop mixtures provide effective sulphate catch crop and sulphur green manure services. Plant Soil. doi: 10.1007/s11104-018-3615-8

Couëdel, A., Alletto, L., Tribouillois, H., Justes, E., 2018. Cover crop crucifer-legume mixtures provide effective nitrate catch crop and nitrogen green manure ecosystem services. Agric. Ecosyst. Environ. 254, 50–59. doi:10.1016/j.agee.2017.11.017

National journals:

Couëdel, A., Seassau, C., Wirth, J., Alletto, L., 2017. Potentiels de régulation biotique par allélopathie et biofumigation ; services et dis-services produits par les cultures intermédiaires multiservices de crucifères. Innov. Agron. 62, 71–85. doi:10.15454/1.517407346984539E12

Tribouillois, H., Bedoussac, L., **Couëdel, A.**, Justes, E., 2017. Acquisition des ressources et production de services écosystémiques par les mélanges bi-spécifiques de cultures intermédiaires. Innov. Agron. 62, 17–32. doi:10.15454/1.5174020133461763E12

Conferences:

Oral presentations:

Couëdel, A., Alletto, L., Tribouillois, H., Kirkegaard, J., Justes, E., 2018. Crucifer-Legume bispecific cover crop mixtures provide efficiently various ecosystem services. European Society for Agronomy Congress. Geneva, 27-31 August.

Couëdel, A., Alletto, L., Justes, E., 2017. Les cultures intermédiaires multi-services en mélanges crucifère/légumineuse combinent des effets de piège à sulfate et "d'engrais vert à soufre" pour la culture suivante. Colloque Soufre INRA. Université de Caen, 12-13 July.

Oral presentation and poster:

Couëdel, A., Alletto, L., Giulinao, S., Perdrieux, F., Rametti, G., Justes, E., 2016. Mixed crucifer–legume cover crops produce efficient nitrogen management ecosystem services. European Society for Agronomy Congress. Edinburgh, 5-9 September. Poster

Poster:

Couëdel, A., Alletto, L., Justes, E., 2018. N and S cycles in crucifer-legume cover crop mixtures. 20th N workshop. Rennes, 25-27 June. doi:10.13140/RG.2.2.33889.40803

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Chapter 1. General introduction

1. Why to sow multi-services cover crop mixtures?

1.1. Abiotic and biotic services linked to cover cropping

Grown between two main cash crops for services production purposes, multi-service cover crops (MSCC) are one of the key tools for sustainable intensification of agriculture (Justes and Richard, 2017; Justes, 2017) (Figure 1). “Nitrate Directive” established by European public policy includes action plans for reducing nitrate leaching and obliged farmers in vulnerable areas to grow cover crops. Surface used for cover cropping increases in France each year and farmers start to see this practice as an opportunity to support agricultural production instead of a simple obligation and a constraint to reduce nitrate leaching. Assessment of the diverse services provided by MSCC is then key to design agroecological production systems (Justes, 2017). The term cover crops was firstly used to refer to services provided linked to soil cover and soil structure enhancement to avoid wind and water erosion (Blanco-Canqui et al., 2015). MSCC can also be called “catch crop” as they catch up nutrients and avoid their losses by leaching limiting economic and environmental issues (Justes et al., 2012). Once cover crops terminated the term “green manure” refers to services of nutrient release for the next cash crop (Thorup-Kristensen et al., 2003). MSCC also provide biotic services to suppress pests, diseases and weeds trough competitions for resources and release of toxic molecules into air and soil (Reddy, 2017). Beyond these services MSCC may also bring disservices as for example biocide effects on beneficials as well as water and nutrient preemption and net organization compared to bare soils (Justes et al., 2012).

A wide range of botanical families are available as MSCC to provide different type or levels of services (Tribouillois et al., 2015). Among species used crucifers are interesting for their efficient catch crop effects due to their fast grow and their strong capacity to catch up nitrate and sulphate (Thorup-Kristensen et al., 2003). Crucifer are also particularly interesting for biocontrol services as they release (during their growing period and once terminated) biocidal hydrolysis products such as isothiocyanates (ITCs) from the secondary metabolites glucosinolates (GSLs) present in their tissues (Matthiessen and Kirkegaard, 2006). Pest,

pathogen and weed suppression by these gaseous ITCs has previously been termed "biofumigation" (Kirkegaard et al., 1993). Crucifer can then be referred as "biofumigants" in comparison to chemical fumigants used for fumigation.

On the other hand, legumes are a key cover crop family as they usually provide the most effective nitrogen green manure service which induces faster mineralisation, and of a larger proportion, of their acquired N present in their residues returning to the soil (Tonitto et al., 2006). Legumes acquired a large proportion of their N through atmospheric N_2 fixation, meaning that exogenous N is added to the agroecosystem (Peoples et al., 1995).

One straightforward way to mutualize services provided by cover crops while mitigating services is the use of plant mixtures (Finney et al., 2017; Tribouillois et al., 2017). Crucifer-legume mixtures could be particularly interesting to mutualize both biotic and abiotic services but studies aiming to assess their performances are scarce.

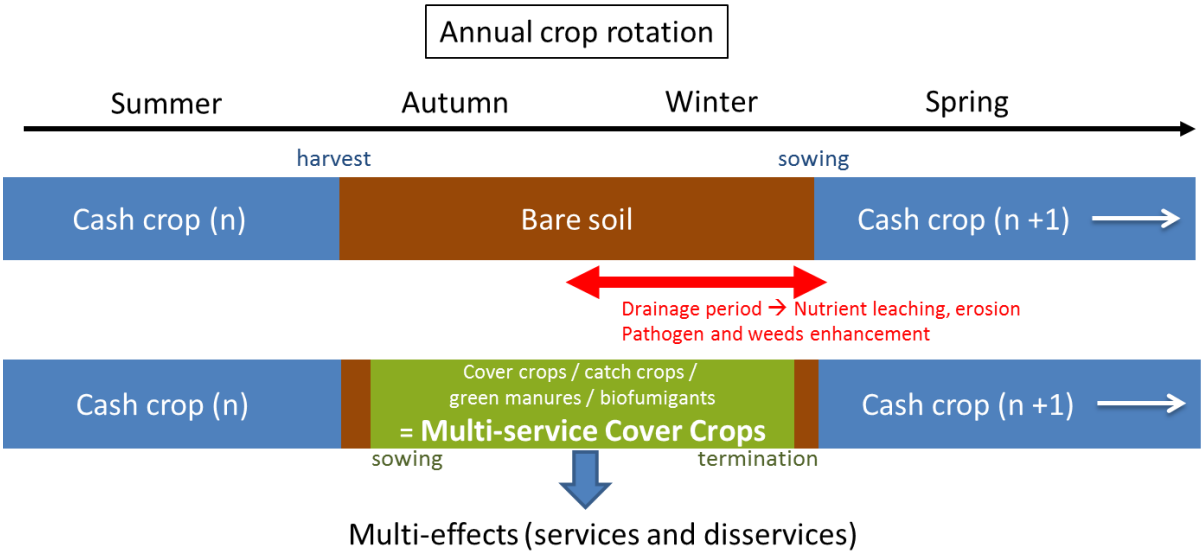


Figure 1. Multiservice cover crops grown in fallow period instead of bare soil to provide services.

1.2. Cover crop mixtures to mutualize services and mitigate disservices?

The main interest of mixtures compared to sole crops is their better resources acquisition through niche complementarity in using abiotic resources such as light, water and nutrients (Tribouillois et al., 2016a). Interestingly, high resources use efficiency of crucifer-legume mixtures has been demonstrated for grain production (Andersen et al., 2005, Fletcher et al 2016), forage crops (Jeromela et al., 2017) and cover crops (Tribouillois et al., 2016; Wendling et al., 2017).

The specific interest of mixtures in cover cropping also reside in the mutualisation of the different services provided by both species. Cover-crop mixtures composed of legume and non-legume species are the most interesting as they could provide both nitrate catch crop and nitrogen green manure services simultaneously by combining advantages of both sole crop species (Kramberger et al., 2009; Rannells and Wagger, 1996; Tosti et al., 2014). One particular interest of crucifer legume mixtures is to achieve biotic services due to the biofumigation potential of crucifers. Furthermore, for general biocontrol, species mixtures are seen as one credible solution to improve pest and diseases suppression while avoiding potential disservices of sole-species compared to sole crops (Boudreau, 2013; Trenbath, 1993). Nevertheless assessment of overall biocontrol potential of crucifer legume mixtures is clearly lacking in the literature.

Despite considerable interest in cover crop mixtures to enhance biotic and abiotic multiservice in agroecosystems few studies have deeply investigated the performances of crucifer-legume mixtures. The main concerns and challenges in designing crucifer legume mixtures is that crucifers could be a poor companion crop for two main reasons: 1) they strongly compete for water, nutrients and light due to their rapid root and shoot growth and 2) they can have an allelopathic effect on legumes due to their production of glucosinolates (Chew, 1988a; Matthiessen and Kirkegaard, 2006). Therefore, screening and identification of crucifer and legume species compatible to design highly performant multiservice mixtures is clearly needed.

2. Objectives and research questions

N and Sulphur (S) cycles are key to manage in fallow period as both nitrate and sulphate are highly leachable through drainage. N cycle services provided by cover crops are well documented but haven’t been widely studied for cover crop mixtures. S availability in cropping system is becoming a major concern but management of its cycle through cover cropping is not well documented. Exogenous S supply has significantly decreased in European systems in the last decades mainly due to reduced S deposition by industries (Scherer, 2001). S cycle is then key to manage in the fallow period to avoid cash crop S deficiency later on in the season. Despite promising interest of crucifer legume mixtures to mutualise services linked to N and S very few studies aimed to understand their level of

performances for a wide range of species. In terms of pathogen and weed suppression, biofumigation potential of crucifer sole cover crops through GSL production remain unclear and knowledge needs to be updated since large broad reviews from Brown and Morra (1997) and Matthiessen and Kirkegaard (2006). Inclusion of legumes could be seen as a way to increase biocontrol potential of crucifer sole cover crops through pathogen disturbance but review studies are lacking to confirm this hypothesis.

The main objective of the thesis is to assess performances and services linked to N, S and biocontrol potential provided by crucifer–legume cover crop mixtures. Specific objectives were to:

1) Update knowledge on biofumigation potential of crucifers and assess interest of crucifer legume mixtures to increase biocontrol.
2) Assess levels of N and S green manure and catch crop ecosystem services provided by crucifers and legumes as sole crops and in mixtures.
3) Understand for a wide range of mixtures the type of interaction between the two species (complementarity or competition) involved in biomass production, N and S acquisition.
4) Compare concentration (plant basis) and production (per basis area) of GSL in crucifer as sole cover crops and mixtures.

3. Methods

This thesis formed part of the CRUCIAL project aiming to assess services provided by crucifers either as sole cover crops or in mixture with a legume. This project gathered academicals partners (INRA, EI Purpan), seed companies and breeders (Jouffray Drillaud and RAGT). CRUCIAL was financially supported by the French Ministry of Agriculture (CASDAR project no. C-2013-05) and the Occitanie Region (CLE project no. 13053068).

To meet objectives 2 to 4 field experiments were conducted at two sites, near Toulouse (France) and Orléans (France) for two years. Few cultivars from eight crucifers (rape, white mustard, Indian mustard, Ethiopian mustard, turnip, turnip rape, radish and rocket) and nine legumes (Egyptian clover, crimson clover, common vetch, purple vetch, hairy vetch, pea, soya bean, faba bean, and white lupin) were tested in sole-crop and in 98 bispecific mixtures (substitutive design of 50%-50% density of sole crops) on a 20 m^2 plots per treatments. Cover crops shoot and roots were harvested 3 months after sowing. Variables collected included

cover crop biomass, nutrient content, GSL production and soil mineral N. Objective 1 were tested thanks to a review analysis of literature on a wide range of pathogens and beneficials. This review was carried out in collaboration with John Kirkegaard in Canberra (CSIRO, Australia) where Antoine Couëdel stayed 3 months. Grant and travel expenses were financed by a CSIRO-INRA linkage program and "Ecole des docteurs" of Toulouse.

4. Thesis outlines

This thesis consists of six chapters: a general introduction (Chapter 1), four research chapters (Chapter 2 to 5), and a general discussion (Chapter 6) (see Figure 2 for details).

Chapter 2 proposes a refocus of biofumigation research that moves from the previous "pesticide" paradigm that targets maximum production of bioactive compounds by sole crucifer cover crops, to a more ecologically-based multi-service paradigm in which cover crop mixtures may offer significant promise (Objective 1).

Chapter 3 assess nitrate catch crop and N green manure services provided by crucifer legume cover crops in comparison with sole cover crops (Objective 2). This chapter include assessment of interactions between the two species (complementarity or competition) involved in N acquisition (Objective3).

Chapter 4 proposes to study S cycle in cover cropping in a similar way than N cycle. We then assess sulphate catch crop and S green manure services provided by crucifer legume cover crops in comparison with sole cover crops (Objective 2). This chapter also include assessment of interactions between the two species (complementarity or competition) involved in S acquisition (Objective 3).

Chapter 5 assess the impacts of crucifer-legume bispecific mixtures on the GSL type, concentration and production at both the individual plant level and on a ground area basis for ecological and agronomic purposes (Objective 4).

Chapter 6 proposes a systemic approach to evaluate biocontrol services provided by cover crops. A multiservice approach framework is also proposed to guide cover crop selection. Gaps of knowledge are highlighted to orient future research efforts on cover cropping.

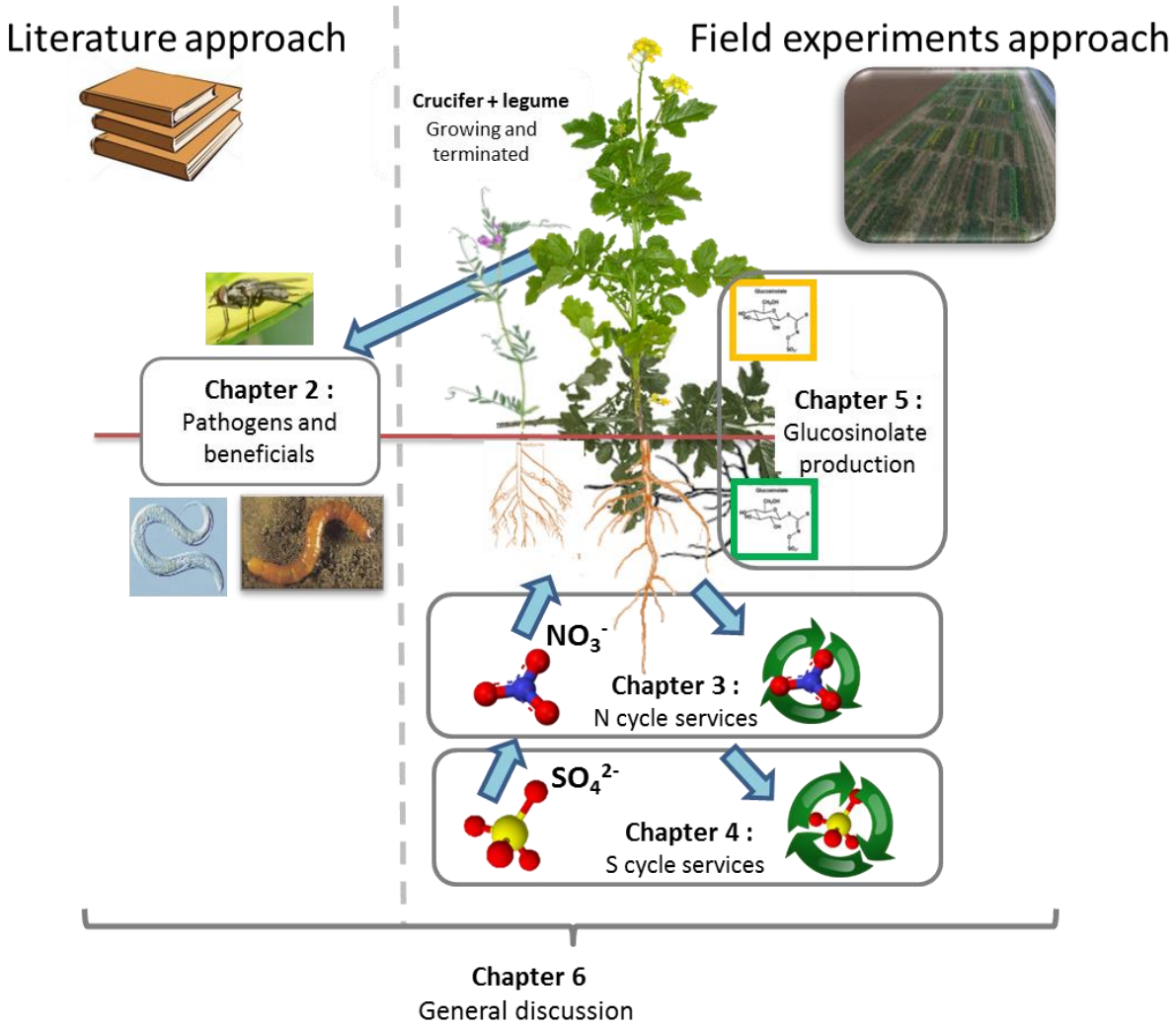


Figure 2. Outline of the thesis. Chapters are either based on literature analysis (2), field experiments (3, 4, 5) or gathered both approaches to propose new insights for future cover cropping research (6).

Chapter 2. Crucifer – legume cover crop mixtures for biocontrol: a new multi-service paradigm.

This chapter correspond to the 2 first parts of a review manuscript submitted in Advances in Agronomy as:

Couëdel, A. Kirkegaard, A. Alletto, J. Justes, E. Crucifer – legume cover crop mixtures for biocontrol: a new multi-service paradigm.

Graphical representation of research objectives:

In this chapter, for pathogens and beneficial for which references were available we ask the questions:

- Does crucifers are an efficient solution for biocontrol without engendering disservices through beneficial suppression?
- Is including mixtures increasing biocontrol services and mitigating disservices?

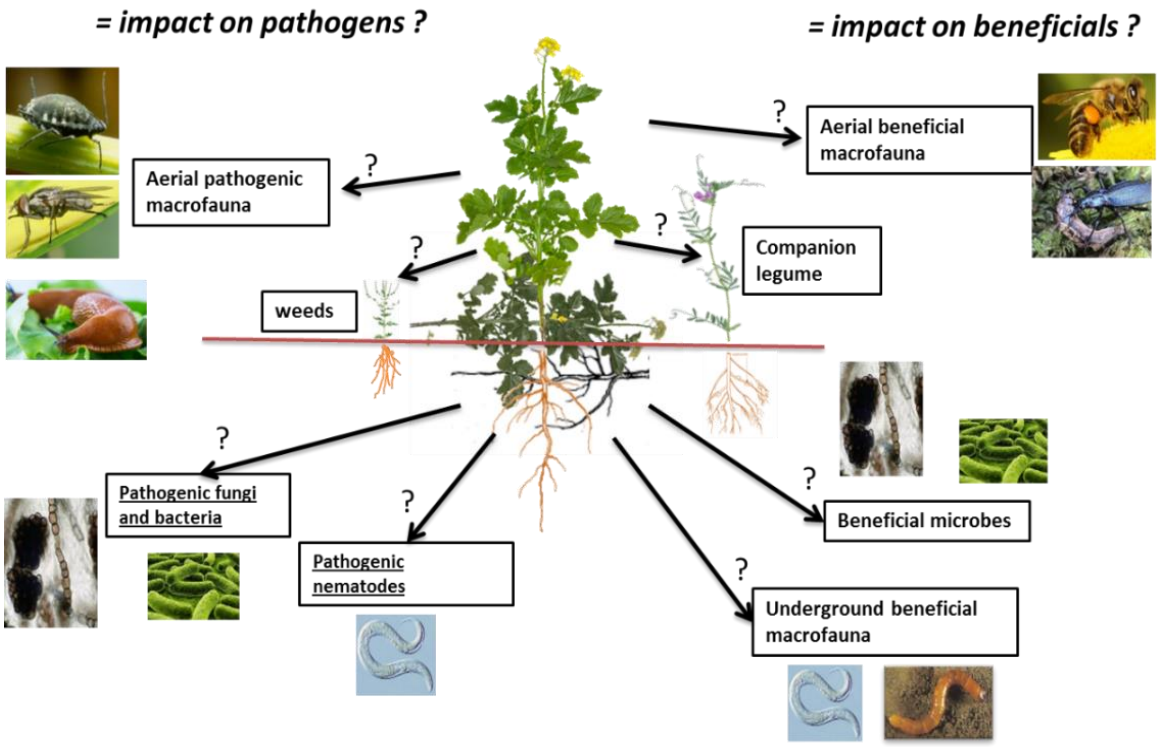


Figure 3. Summarized scheme of questions raised by the chapter 2

Introduction

Improving plant diversity in cropping systems in both space and time can enhance ecosystem services while mitigating the potential disservices generated by anthropogenic inputs (Duru et al., 2015; Gaba et al., 2015). Usually grown between two cash crops in an annual rotation, cover crops can increase diversity and provide a number of ecosystem-services. The notion of cover crops has evolved historically since the last century where it was largely developed in US agriculture (e.g. Meisinger et al., 1991) for erosion control or green manuring, but now encompasses other benefits such as biocontrol and catch crops (Justes, 2017). Recently the new paradigm of multi-services cover crops (MSCC) has been proposed to capture these multiple benefits using principles of agroecology (Justes and Richard, 2017). The MSCC are specifically grown in the fallow period between two main cash crops, are not harvested and their biomass remains on or in the soil. They are neither forage crops nor harvested for biogas. The potential provision of multi-services depends on the species or mix of species chosen, the management of the cover crop, the pedo-climatic conditions and the cash crop sequence involved. The potential services include i) nitrogen management, i.e. nitrate catch crop (e.g. Justes et al., 1999; Constantin et al., 2011; Thomsen & Hansen, 2014; Couëdel et al., 2018) and green manuring (e.g. Thorup-Kristensen et al. 2003; Tonito et al., 2006; Tribouillois et al., 2016), ii) nutrient cycling in general (Thorup-Kristensen et al., 2003) including for specific anions such as sulfate (Couëdel et al., 2018); iii) soil protection and improvement of soil physical properties (e.g. Blanco-Canqui et al., 2015; Justes et al., 2012), iv) organic matter and carbon storage in the soil (Justes et al., 2012; Constantin et al., 2011), v) reduced direct greenhouse gas emissions to mitigate climate change (Poeplau and Don, 2015; Tribouillois et al., 2018), and vi) suppression of weeds (e.g. Teasdale, 1996) and pathogens (e.g. Farooq et al., 2011; Reddy, 2017). In some cases, the MSCC can also contribute to ecological services such as plant pollination, maintenance of wild fauna (birds, small mammals, insects, etc.) and contribute to landscape aesthetics (Justes et al., 2017).

No cover crop or cover crop mixture will provide all of these services, and some may generate disservices such as pre-emptive competition for water and nutrients with subsequent cash crops (Thorup-Kristensen and Kirkegaard 2016). Consideration of the potential trade-offs is essential in choosing species or species mixtures as many botanical families can be used (cruciferous, grass, legume, other families). Clearly avoiding species that may act as hosts to important diseases and pests of the main cash crop is advisable but other less obvious

interactions between species in the cover crop, and between cover crop and main crop can occur. Indeed several recent studies highlight the important genotype-environment-management interactions that underpin the success of cover crop choices (Lemessa and Wakjira, 2015; Schipanski et al., 2014; Snapp et al., 2005, Farooq et al 2011). Here we focus on the potential contribution of MSCC for biocontrol in future cropping systems to reduce the use of pesticides in the sustainable intensification of agriculture.

Among cover crop species commonly used worldwide (Table 1), Brassicaceae or crucifer cover crops are especially interesting for pathogen and weed suppression due to the release of several potentially biocidal hydrolysis products such as isothiocyanates (ITCs) from the secondary metabolites known as glucosinolates (GSLs) present in their tissues (Brown and Morra, 1997; Van Dam et al., 2009). Crucifer cover-crops can generate GSL-related biocidal effects both during the growing period when hydrolysis products such as ITCs are released in the rhizosphere around the growing roots (McCully et al., 2008; Rumberger and Marschner, 2004**),** or when tissue disruption occurs following cover crop termination and incorporation (Matthiessen and Kirkegaard, 2006) (Figure 4). Pest, pathogen and weed suppression by ITCs via these two processes has previously been termed "biofumigation" (Kirkegaard et al., 1993), and has generated interest from both an agronomic and an ecological perspective in regulating soil biological communities (Bressan et al., 2013), including fungi (Motisi et al., 2009), bacteria (Arthy et al., 2002), nematodes (Fourie et al., 2016), and weeds (Bangarwa and Norsworthy, 2016; Jabran et al., 2015). At the end of the 20th century, biofumigation was seen as a potential option to assist in the replacement of banned chemical fumigants widely used in horticulture including methyl bromide (MeBr), and it was thought to be one of the most promising non-chemical control options at the time (Porter et al., 1999). However the levels of pathogen suppression achieved under field conditions using biofumigant cover crops alone is usually less than that achieved with fumigant chemicals, as the level of ITCs found in soil with biofumigants is much lower than that achieved with chemical fumigants such as methyl-isothiocyanate (Gimsing and Kirkegaard, 2009). The levels achieved and their effectiveness are also influenced by the sensitivity of the pathogen of interest, but also the soil type, temperature and organic matter levels influence the activity of ITCs in soil (Gimsing and Kirkegaard, 2009). Approaches to link individual ITC release to the suppression of specific pests and pathogens have been explored in numerous controlled laboratory experiments (Brown and Morra, 1997), but less so under field conditions where many other effects of cover crops aside from ITC-related

suppression are operating (Matthiessen and Kirkegaard 2006). As for the chemical fumigants such as MeBr, GSL-related biocides can also generate potentially negative effects on beneficial microbe and insect communities (Omirou et al., 2011), but these disservices and their relevance have had less focus to date within the research literature. Crucifer cover crops may also have other undesirable characteristics as cover crops such as hosting pathogens (e.g. Pythium species) or acting as non-hosts to beneficial organisms such as mycorrhizae in some systems (Ahuja et al., 2010).

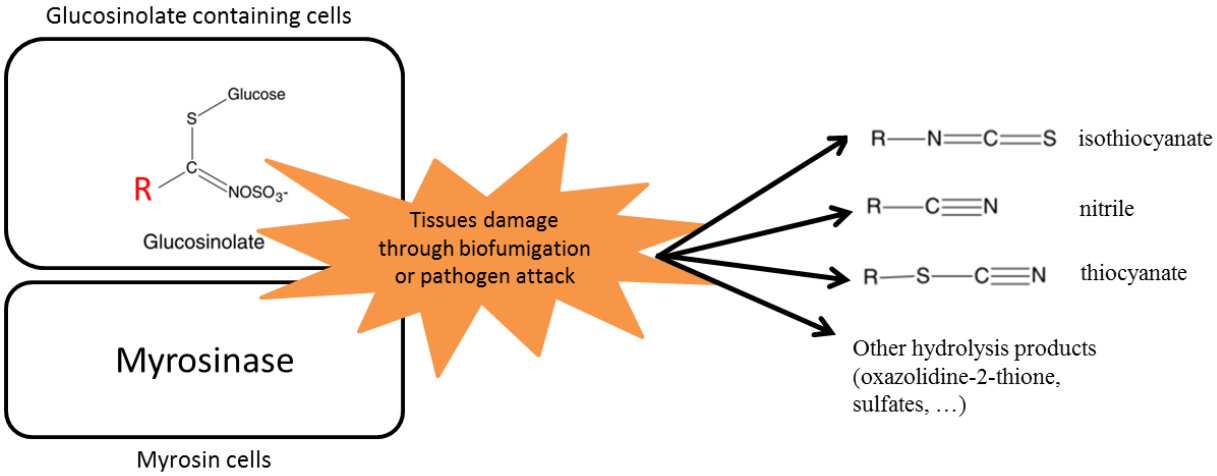


Figure 4. Hydrolysis and main degradation products of glucosinolates including the biocidal isothiocyanates.

One possible solution to improve pest and diseases suppression while avoiding potential disservices of sole-species cover crops may be to use species mixtures to provide better suppression of certain pests, diseases and weeds compared to sole crops (Trenbath, 1993; Boudreau, 2013). In particular, crucifer-legume substitutive bi-specific mixtures (i.e. 50% of each species in a mixture) have been found to outperform crucifer sole crops in terms of multiservice benefits as harvested cash crops (Fletcher et al., 2016), forage crops (Jeromela et al., 2017) or nitrate catch crops and green manures (Couëdel et al., 2018c) due to an improved biomass production (see examples of crucifer legume mixtures in Figure 5). Legumes can provide nitrogen input through N fixation (Thorup-Kristensen et al., 2003). Crucifer-legume mixtures can also achieve better resource use efficiency, due to the niche complementarity in using abiotic resources such as light, water and nutrients (Jensen, 1996) and have also been shown to increase the amount of N mineralized for the next cash compared to crucifer sole crops (Tribouillois et al., 2016a).

Figure 5. Crucifer-legume mixtures used in diverse production systems. (A) Turnip rape - Egyptian clover cover crop mixture in France (© Antoine Couëdel), (B) Pea intercropped with canola in Australia (© John Kirkegaard), (C) service legumes (common vetch and lentil) intercropped with oilseed rape in France (© Terre-net Media), (D) Broccoli vegetable production intercropped with crimson clover in the US (© Mark Schonbeck).

Despite considerable interest in cover crop mixtures to support the concept of MSCC, few studies have investigated the impact of crucifer-legume mixtures on the suppression of pathogens and weeds. Understanding species choice to successfully combine the recognised nutrient cycling benefits of legumes with the pest suppressive potential of crucifers will be crucial to design appropriate mixtures that can achieve useful pest suppression without generating related disservices within the agroecosystem. Diversity for its own sake in agricultural systems has been called into question (Ford Denison and McGuire, 2015), but where multi-services can be demonstrated and managed in an agronomic sense, the concept can assist sustainable intensification. Our purpose was to investigate the potential to move from a largely "pesticide" paradigm that targets maximum production of bioactive compounds by sole crucifer cover crops, to a more multi-service paradigm where cover crop mixtures may assist sustainable intensification of agriculture. We firstly update the knowledge of the biocontrol potential of crucifer species used as cover crops, both in terms of the services and potential disservices. We update and build upon the previous reviews of

Matthiessen and Kirkegaard (2006); Kirkegaard et al, (2009) and Motisi et al., (2010) and focus on the mechanisms behind pathogen and weed suppression by crucifer cover crops. We then consider how crucifer-legume bispecific mixtures could enhance pathogen and weed suppression and minimise disservices compared to crucifer sole crops by considering the main services and disservices reported for microbial, weed, nematode and macro-faunal suppression. We conclude by considering research approaches to fill the considerable knowledge gaps in this area, and strategies to improve cover crop species selection and breeding toward a multiservice approach.

Table 1. Common names, family and species for the crops and cover crops mentioned in this review

Common name	Family	Latin name
Alfalfa	Legume	*Medicago sativa L.*
Arabidopsis	Crucifer	*Arabidopsis thaliana* L.
Beetroot	Chenopodiaceae	*Beta vulgaris L.*
Black mustard	Crucifer	*Brassica nigra* (L.) W.D.J. Koch
Bristle oat	Cereal	*Avena strigosa,* Schreb.
Broccoli	Crucifer	*Brassica oleracea var. italica (L.) Plenck*
Buckwheat	Polygonaceae	*Fagopyrum esculentum* Moench,
Bigpod sesbania	Legume	*Sesbania herbacea* (Mill.) McVaugh
Cabbage	Crucifer	*Brassica oleracea L.*
Camelina	Crucifer	*Camelina spp.*
Canola, rape	Crucifer	*Brassica napus L.*
Cauliflower	Crucifer	*Brassica oleracea* var. *botrytis L.*
Common vetch	Legume	*Vicia sativa* L.
Cotton	Malvaceae	*Gossypium hirsutum* L.
Cowpea	Legume	*Vigna unguiculata* (L.) Walp.
Creeping thistle	Asteraceae	*Cirsium arvense L.*
Crimson clover	Legume	*Trifolium incarnatum L.*
Crownvetch	Legume	*Coronilla varia* L.
Egyptian clover	Legume	*Trifolium alexandrium L.*
Faba bean	Legume	*Vicia faba L.*
Hairy vetch	Legume	*Vicia villosa* Roth.
Indian mustard	Crucifer	*Brassica juncea L. Czern.*
Joint vetch	Legume	*Aeschynomene americana* L.
Kura clover	Legume	*Trifolium ambiguum* M.B
Linseed	Linaceae	*Linum usitatissimum L.*
Lupin	Legume	*Lupinus luteus L.*
Mashua	Tropaeolaceae	*Tropaeolum tuberosum* Ruiz & Pavon
Pea	Legume	*Pisum sativum L.*
Pennycress	Crucifer	*Thlaspi arvense* L.
Potato	Solanaceae	*Solanum tuberosum L.*
Radish	Crucifer	*Raphanus raphanistrum subsp. Sativus*
Red clover	Legume	*Trifolium pratense* L.
Rocket	Crucifer	*Eruca sativa* Mill.
Rye	Cereal	*Secale cereale L.*
Snapbean	Legume	*Phaseolus vulgaris L.*
Sorghum-sudangrass	Cereal	*Sorghum ×drummondii* (Nees ex Steud.) Millsp. & Chase
Soybean	Legume	*Glycine max* (L.) Merr.
Subterranean clover	Legume	*Trifolium subterraneum* L.
Sunn hemp	Legume	*Crotalaria juncea* L.
Tall fescue	Cereal	*Festuca arundinacea* Schreb.
Tobacco	Solanaceae	*Nicotiana tabacum L.*
Tomato	Solanaceae	*Solanum lycopersicum L.*
Trefoil	Legume	*Lotus corniculatus L.*

Turnip	Crucifer	*Brassica rapa L. subsp. Rapa*
Turnip Rape	Crucifer	*Brassica rapa subsp. Rapa*
Velvet bean	Legume	*Mucuna pruriens* (L.) DC.
Watermelon	Cucurbitaceae	*Citrullus lanatus* (Thunb.) Matsum. & Nakai
Wheat	Cereal	*Triticum* L.
White cabbage	Crucifer	*Brassica oleracea* var. *capitata*
White clover	Legume	*Trifolium repens* L.
White mustard	Crucifer	*Sinapis alba L.*
Wild mustard	Crucifer	*Sinapsis arvensis* L.
Wintercress	Crucifer	*Barbarea vulgaris R.Br.*

Table 2. Latin names and abbreviations used for microbes mentioned in this review

Latin name	Abbreviation used
Rhizoctonia solani	*R. solani*
Verticilium dahliae	*V. dahliae*
Gaeumannomyces graminis var. tritici	*G. tritici*
Aphanomyces euteiches	*A. euteiches*
Ralstonia solanacearum	*R. solanacearum*
Streptomyces scabies	*S. scabies*
Fusarium oxysporum	*F. oxysporum*
Fusarium graminearum	*F. graminearum*
Fusarium chlamydosporum	*F. chlamydosporum*

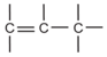
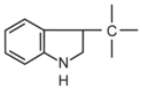
Table 3. Latin names and abbreviations used for nematodes mentioned in this review

Latin name	Abbreviation used	Type of nematode
Meloidogyne incognita	*M. incognita*	root-knot nematode
Meloidogyne hapla	*M. hapla*	root-knot nematode
Meloidogyne arenaria	*M. arenaria*	root-knot nematode
Meloidogyne incognita	*M. incognita*	root-knot nematode
Meloidogyne javanica	*M. javanica*	root-knot nematode
Meloidogyne chitwoodi	*M. chitwoodi*	root-knot nematode
Paratrichodorus allius	*P. allius*	cyst nematodes
Globodera rostochiensis	*G. rostochiensis*	potato cyst nematode
Globodera pallida	*G. pallida*	potato cyst nematode
Heterodera schachtii	*H. schachtii*	beet cyst nematode
Pratylenchus neglectus	*P. neglectus*	root-lesion nematode
Pratylenchus penetrans	*P. penetrans*	root-lesion nematode
Belonolaimus longicaudatus	*B. longicaudatus*	sting nematodes
Caenorhabditis elegans	*C. elegans*	beneficial nematode

Table 4. Latin names and abbreviations used for insects mentioned in this review

Latin name	Abreviation used	Common name
Delia radicum	*D. radicum*	Cabbage root fly
Plutella xylostella	*P. xylostella*	Diamondback Moth
Phyllotreta cruciferae	*P. cruciferae*	Crucifer flea beetle
Dasineura brassicae	*D. brassicae*	*Brassica* pod midge
Ceutorhynchus picitarsis	*C. picitarsis*	rape winter stem weevil
Pieris rapae	*P. rapae*	cabbageworm fly
Acyrtosiphon pisum	*A. pisum*	Pea aphid
Cylas formicarius	*C. formicarius*	Potato weevil
Delia floralis	*D. floralis*	Turnip root fly

Table 5. Most common glucosinolates found in crucifer cover crops. Examples of cover crops are not exhaustive and are derived from Couëdel et al., (2018b). *Side chain correspond to the "R group" mentionned in Figure 2.

Side chain*	Chemical name	Common name	Example of presence in shoots	Example of presence in roots
Aliphatic	2-Propenyl	Sinigrin	Ethiopian and Indian mustard	Ethiopian and Indian mustard
	4-Methylthiobutyl	Glucoerucin	White mustard, rocket	Rape, rocket
	4-Methylsulphinylbutyl	Glucoraphanin	Turnip rape, rocket	Rocket
	4-Methylsulfinyl-3-buteny	Glucoraphenin	Radish	/
	4-Methylthio-3-butenyl	Glucoraphasatin	Radish	Radish
	3-Butenyl	Gluconapin	Turnip rape, rocket	Turnip rape
	2-Hydroxy-3-butenyl	Progoitrin	Turnip rape	Turnip, turnip rape
	4-Pentenyl	Glucobrassicanapin	Turnip rape	Turnip rape
	2-Hydroxy-4-pentenyl	Gluconapoleiferin	Turnip	Turnip
	5-Methylsulphinylpentyl	Glucoalyssin	Rape	/
Aromatic	4-Hydroxybenzyl	Sinalbin	White mustard	White mustard
	2-Phenylethyl	Gluconasturtiin	Rape, Turnip rape	Rape, Indian mustard, turnip rape
	Benzyl	Glucotropaeolin	White mustard	White mustard
Indole	4-Hydroxy-3-indolylmethyl	Hydroxyglucobrassicin	Turnip	Turnip
	3-Indolylmethyl	Glucobrassicin	Rape, Ethiopian mustard, radish	Ethiopian mustard, turnip rape
	4-Methoxy-3-indolylmethyl	4-methoxyglucobrassicin	Turnip	/
	1-Methoxy-3-indolylmethyl	Neoglucobrassicin	Rape, Turnip, Turnip rape	Rape, Indian mustard, turnip rape

1. Mechanisms of pathogen and weed suppression by crucifer sole crops and mixtures

The impact of crucifer cover crops on pathogens, beneficial organisms and weeds can arise not only from allelochemical effects, but from numerous other processes such as non-hosting effects, plant cover effects, organic matter addition and soil structural effects (Figure 6). In this first section we review the conceptual interest of cover crop mixtures as tools to improve suppression of pathogens and weeds while minimising potential disservices on beneficial organisms. In each section we focus first on crucifer sole crop effects, and then consider the likely changes associated with the inclusion of a legume with the crucifer in a mixture. Figure 3 provides a schematic summary of the concepts discussed in this section. All latin names and abbreviations of crops, microbes, nematodes and insects mentioned in this review are displayed in Table 2, Table 3 and Table 4.

1.1. Allelochemical production

There are about 132 different GSLs commonly found in crucifer's spp. that vary in their structure depending on the character of the organic side chain (aliphatic, aromatic or indole) (Table 5). Present in plant vacuoles, GSLs generate toxic hydrolysis products when they react with the enzyme myrosinase through tissues damage due to biofumigation or pathogen attack (Figure 4) (Andréasson and Jørgensen, 2003; Kissen et al., 2009). Biocidal molecules produced are isothiocyanates (ITCs), thiocyanates and nitriles (Figure 4) (Brown and Morra, 1997). Aliphatic GSLs produce the most volatile ITCs due to a short organic side chain while aromatic ITCs are in general less volatile but have a higher contact toxicity as they are more lipophilic to penetrate live membranes (Matthiessen and Kirkegaard, 2006). Indole GSLs do not produce ITCs and are generally considered less toxic than other GSL types (Fahey et al., 2001). GSL types and concentrations vary among crucifer species, and between root and shoot tissues of the same species (Bellostas et al., 2007; Couëdel et al., 2018b; Kirkegaard and Sarwar, 1998). Beyond GSL production, crucifers also produce other compounds that may be biologically active such as S-containing methanethiol and dimethyl sulfide (Bending and Lincoln, 1999; Gamliel and Stapleton, 1993) or fatty acid and phenolic molecules (Brown and Morra, 1997). By reacting with GSL by-products such as H_2S, these latter's molecules could improve and prolong biocidal effects (Brown and Morra, 1997). The biofumigant potential of various crucifer cover crops has been previously reviewed

(Matthiessen and Kirkegaard 2006; Motisi et al., 2010) in sole crops but not when used in mixtures.

Bispecific crucifer-legume cover crop mixtures will firstly change the diversity of allelochemicals produced (Figure 6). Indeed legumes themselves can produce a wide diversity of allelochemicals, such as phenolic acids or terpenoides, that differ among species (Mondal et al., 2015; Wink, 2013a). Secondly, plant-plant interactions in mixtures can influence allelochemical concentrations and potentially decrease their biocidal effects. In mixtures, crucifers usually compete strongly with legumes for water, nutrients and light due to their rapid root and shoot growth (Kristensen and Thorup-Kristensen, 2004). Consequently on an individual plant basis, crucifers have been shown to produce higher biomass (S. E. Wortman et al., 2012), to acquire more N (Tribouillois et al., 2016; Wendling et al., 2017) and more S (Couëdel et al., 2018a; Génard et al., 2017) when grown with a legume in a mixture than in a sole crop. As N and S are both essential components of GSLs, Couëdel et al. (2018b) showed that crucifers produced more GSL per plant in mixtures compared to sole crops, but retained the same GSL types and concentration on a dry weight basis. This led to an equivalent level of protection from pests for the crucifer component of the cover crop in the mixture as for the sole crop. In contrast, for the legume component, the high competition from the crucifer could potentially reduce the allelochemical concentration and may lead to lower pest protection. These concerns are important to take into account because pests and diseases of the legume component could lead to lower biomass and hence reduce the ecosystem services associated with nitrogen and carbon cycles. It is possible that allelochemicals released by crucifers in the mixture may also protect the legume from pests or diseases to maintain the multi-services provided. On an area basis, as crucifer density is halved in the bispecific crucifer-legume mixtures, a 50% decrease in crucifer allelochemical production could be predicted (Figure 6). However Couëdel et al., (2018b) showed that for a wide range of crucifer and legume species in mixtures, the GSL production per area was on average only reduced by 19%, thus largely retaining the pest-suppressive capacity of the crucifer within the mixture. There are no equivalent studies investigating the impact of plant-plant interaction on the allelolechemical production of legumes on an area basis. However in crucifer-legume mixtures, the effects of higher allelochemical diversity (cocktail effect) coupled with a somewhat lower production of individual and total GSL on living organisms warrants further investigation in order to assess the multiservice provision by the mixtures.

1.2. Non-host / Host / Trap effects

The fallow period between two cash crops is a key period to break the cycles of pathogens that can't survive long without a suitable host and weeds. Cover crop species are also susceptible to pathogens and must be chosen to avoid the hosting of pathogens that would otherwise decline during bare fallow periods. Crucifers are break crops, or non-hosts, of numerous pathogens (Angus et al., 2015) although despite their release of toxic compounds, they are also host or moderate host of some fungal pathogens (Lu et al., 2010), and nematodes (Ntalli and Caboni, 2017) which can reduce their effectiveness as cover crops in certain conditions. Crucifers are also non-hosts of important beneficial organisms such as mycorrhizal fungi which can be a disservice compared to other cover crops families where mycorrhizal-dependant cash crops follow in the crop sequence (Gosling et al., 2006). Crucifers have also been shown to act as trap crops where they can attract certain pest species such as nematodes (Kruger et al., 2013; Melakeberhan et al., 2006a) and insects (Ahuja et al., 2010; Badenes-Perez et al., 2004), but interfere with the feeding or reproductive cycles to reduce pest populations.

Cover crop mixtures can provide a potential solution to host plant issues of crucifers via several mechanisms (Figure 6). Firstly a dilution effect occurs as the density of crucifers are halved in bispecific mixtures, making the pest less able to locate and infest its host plants (Boudreau, 2013; Root, 1973). Secondly, a physical and visual barrier effect is enhanced by the companion crop by modifying the architecture of the stand, the pest or pathogen spread can then be disrupted as well as the energy required by insects or nematodes in the search for the host plant (Ratnadass et al., 2012; Vandermeer, 1989). Thirdly, a change in host plant morphological traits can result from plant-plant interactions in mixtures to diminish the ability of the pest to find a host (Ratnadass et al., 2012). Finally a change in host susceptibility and attractiveness to pests can also result from plant-plant interaction by changing the host plant chemical composition, such as the production of repellent chemicals or flavour changes (Ratnadass et al., 2012).

The same mechanisms can also reduce the pathogen host effects of legumes, reducing diseases in the legume and generating improved N-related services. However special care must be taken when designing appropriate mixtures as legumes can also host numerous pests and diseases (Ahuja et al., 2010; Ntalli and Caboni, 2017) generating undesirable changes to the pathogen host status of the cover crop. The trap crop effects of crucifer cover crops on

nematodes or insects could also be reduced in mixtures due to the legume component, but no studies are available on this phenomenon.

1.3. Plant cover effects via abiotic resource use

Compared to a bare soil, the presence of a growing plant cover affects light, nutrient and water fluxes as well as microclimate resulting in changes in organism communities (Vukicevich et al., 2016). Through root exudation and rhizodeposition processes, growing cover crops provide large amounts of nutrients to attract and sustain microorganisms including bacteria, non-pathogenic *Fusarium* species, *Streptomyces* and other actinomycetes (Hinsinger et al., 2009; Wichern et al., 2007). Microbial communities have been reported to suppress numerous pathogens through competition, antibiosis, and parasitism or by inducing systemic plant resistance (Audenaert et al., 2002; Rayns and Rosenfeld, 2006). As each plant family and species of cover crop specifically shape the structure of microbial communities (Buyer et al., 2010) through different exudate composition quantity and seasonality (Broeckling et al., 2008; Schweitzer et al., 2008), cover crop species mixtures could enhance soil organism diversity and activity (Berg and Smalla, 2009; Lawrence et al., 2012; Legay et al., 2014) to increase disease suppression (Bardgett and Van Der Putten, 2014; Garbeva et al., 2004). In that regard, crucifer and legumes are complementary because as sole crops they enhance different specific pathogen-suppressive microorganisms (Berg et al., 2005; Vukicevich et al., 2016). Furthermore, complementarity of resource use and root architecture between crucifers and legumes can lead to better root biomass and density in the mixture (Couëdel et al., 2018c) that may generate more exudation and rhizodeposition (Figure 3) (Duchene et al., 2017). In mixtures, this combination of 1) higher root tissue diversity and 2) higher root biomass and density leads to increased microbial diversity and abundance in the rhizosphere (Duchene et al., 2017; Zak et al., 2003) and as a consequence improved control of pests and diseases may result (Latz et al., 2015, 2012).

Plant cover is also key to weed suppression due to competition for abiotic resources (Teasdale, 1996). Crucifer cover crops can compete strongly with weeds for nutrients, light and water due to their rapid increase in root depth and density (Thorup-Kristensen, 2001; Thorup-Kristensen et al., 2003) which allows them to capture a large amount of nitrogen and sulphur soon after sowing. Nevertheless competition for these abiotic resources could be increased in mixtures compared to pure crops thanks to a complementarity in nutrient uptake and light interception as shown previously for cereal-legume mixtures (Corre-Hellou et al.,

2011; Hauggaard-Nielsen et al., 2001). Both weed biomass and diversity can be reduced by some crop mixtures compared to sole crops (Figure 6) (Poggio, 2005).

1.4. Organic matter addition

At cover crop termination, some studies report similar levels of pathogen suppression between crucifers and other cover crop species (Larkin, 2013) that could be due to the effect of organic matter addition rather than specific allelochemical effects (Zhou and Everts, 2007). Pathogen suppression by organic inputs has mostly been attributed to indirect effects of higher antagonist diversity and density rather than a decrease in pathogen inoculum (Davis et al., 1996; Ennaïfar et al., 2005). Indeed organic inputs can improve the soil biological status by increasing both the diversity and the size of beneficial species population through physico-chemical changes (Blanchart et al., 2006; Ochiai et al., 2008; Omirou et al., 2011; Sekiguchi et al., 2007). This biocontrol due to organic matter addition has been shown to last longer than the effects of specific allelochemicals such as ITCs, and is often complementary with allelochemical suppression (Cohen and Mazzola, 2006; Mazzola et al., 2007; Motisi et al., 2009).

Interestingly, high organic matter accumulation from diversified biomass also results in higher microfaunal diversity, particularly that of decomposer taxa, at the expense of herbivore/root-feeding species (Brévault et al., 2007; Brown and Oliveira, 2004). By increasing both shoot and root total biomass production (Couëdel et al., 2018c) as well as tissue diversity, incorporated cover crop mixtures could enhance a more diverse and higher abundance of microbial communities (Figure 6) (Buyer et al., 2010; Ratnadass et al., 2012). Indeed crucifers and legumes enhance different microbes (Vukicevich et al., 2016) as the different quality of the plant litter can influence both the activity and microbial community structure (Bastian et al., 2009; Fanin et al., 2016). This can alter the suppression of soil borne diseases (Van Bruggen and Grunwal, 1996). High quality plant litter (lower C/nutrient ratio) such as that generated by legumes can attract copiotrophic microbes such as *Pseudomonas* while fungal decomposer or actidobacterias are enhanced by low quality litter decomposition (Bastian et al., 2009). We can hypothesise that a greater diversity and number of microbes in the soil would increase the likelihood of antagonism to pathogens (Altieri, 1999). Nevertheless there is a lack of specific studies on the decomposition rate of litter from cover-crop mixtures, the impact on soil microorganisms and disease suppression, and the mechanisms are likely to be complex.

1.5. Soil structural effects

Poor physical soil conditions such as inadequate drainage, poor aggregate stability and high soil compaction can exacerbate the damage from soil-borne diseases (Abawi and Widmer, 2000; Hossain et al., 2012), and weeds (Gardarin et al., 2010) and decrease mycorrhizal root colonization (Homma et al., 2012). During the period of cover crop growth, above-ground plant parts can protect soil aggregates from erosion and direct rain-drop impact while the roots can decompact the soil (Han et al., 2016), and enhance the populations of micro- and macro-faunal organisms such as earthworms (Blanco-Canqui et al., 2015). Furthermore, at cover-crop termination, cover crops can increase organic matter content to improve soil structure (Blanco-Canqui et al., 2015). Compared to other graminaceous cover crops species that have fibrous roots, the interest of crucifers is their deep taproots that can alleviate soil compaction by penetrating compact layers across different soils (Chen and Weil, 2010; White et al., 2016), and the fine surface root systems that can improve aggregation (Bodner et al., 2014; Chan and Heenan, 1996).

Crucifer - legume cover crop mixtures with complementary rooting systems (e.g. deep and shallow) have potential to improve soil structure by decreasing soil compaction more effectively than pure crops (Figure 6) (Blanco-Canqui et al., 2015; White et al., 2016). The success of mixtures nevertheless depends on soil conditions, and in some cases soil physical properties are not improved (Fernandes et al., 2011). Planting a mix of species having different shoot architecture can also provide more soil cover (S. E. Wortman et al., 2012), and thereby reduce wind and water erosion (Chen and Weil, 2010).

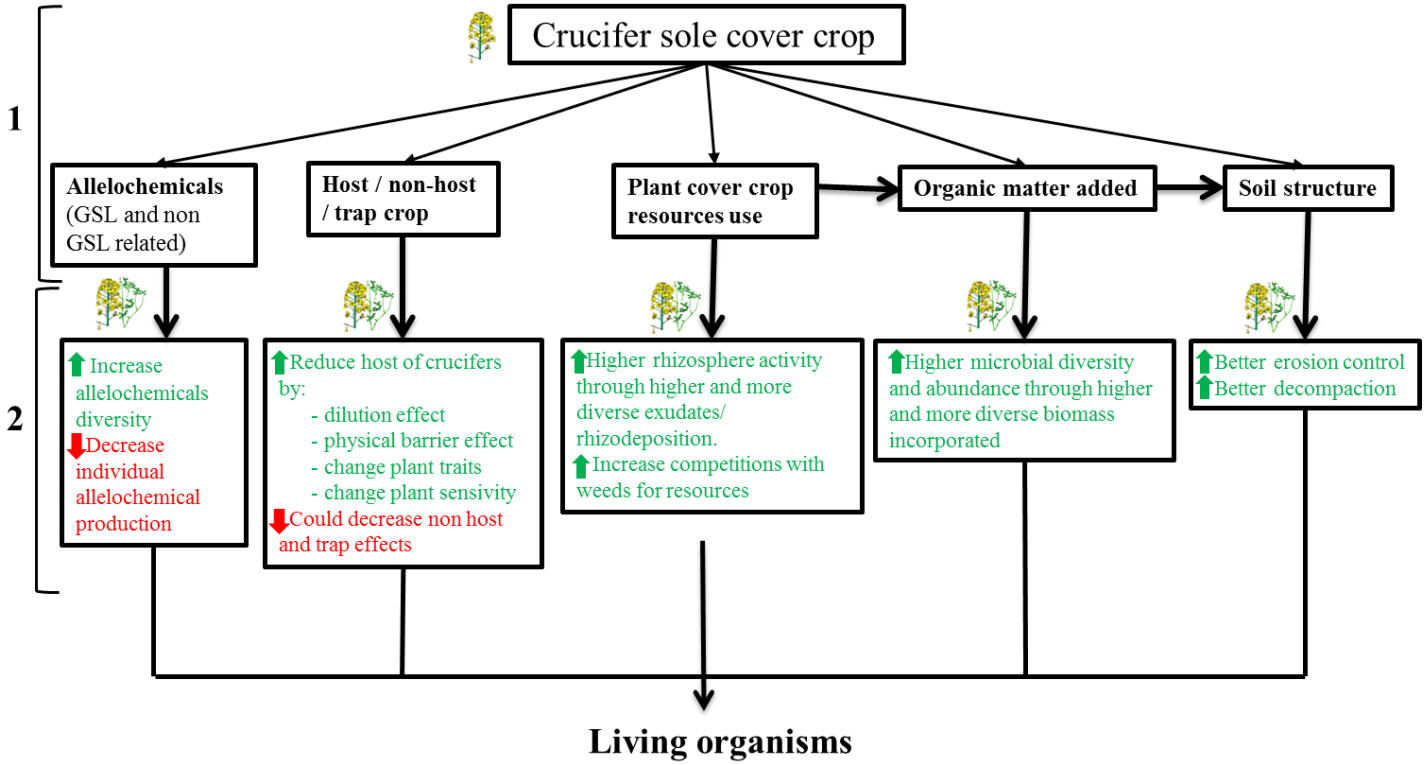


Figure 6. Mechanisms by which (1) crucifer cover crops can influence biocontrol of soil organisms, and (2) how these are potentially affected within a crucifer-legume mixture including services (up-arrow) and disservices (down-arrow) for pathogen suppression.

2. Ecological services and disservices of crucifer sole crops and crucifer legume mixtures

In this section, we review studies dealing with the impact of crucifers as sole cover crops and in mixtures on a wide range of living organisms. Pathogen suppression as well as impacts on beneficial organisms are discussed as they are both important processes within the system. In each subsection, we first discuss the evidence for *in vitro* toxicity of GSL-hydrolysis products on various organisms and then consider field experiments that include crucifer sole cover crops. Where possible we include studies where the inclusion of legumes in mixtures was considered, but in the absence of published literature we speculate about the possible interactions. As a guide to the discussion, Figure 7 provides a summary of the effects of crucifer sole crops and mixtures on each of the living organism classes considered.

2.1. Impact of crucifer cover crops on microbial communities

2.1.1. Pathogen suppression

In *in vitro* studies, ITCs released by crucifers have demonstrated promising biocidal effects on numerous pathogenic microbes including *Rhizoctonia solani*; *Verticilium dahliae*; *Gaeumannomyces graminis var. tritici*, *Aphanomyces euteiches and Ralstonia solanacearum* (Brown and Morra, 1997; Neubauer et al., 2014; Seassau et al., 2016; Smith and Kirkegaard, 2002). In field conditions or in soil, suppression has often been more variable between studies and depends to a significant extent on the soil type, climate and incorporation practices used as these all influence the concentration of allelochemicals and their effectiveness in soil (Matthiessen and Kirkegaard, 2006; Motisi et al., 2010). As highlighted in Part 1, many mechanisms can be involved and can vary according to the specific pathogen considered.

For example, incidence of *R. solani* on beetroot was reduced by 45% by Indian mustard cover crops (Motisi et al., 2009) while it has been reduced by rape (-65%), and white mustard (-45%) but not by Indian mustard on potato (Larkin and Griffin, 2007; Little et al., 2004). In these studies, suppression was more effective by incorporating the cover crops rather than removing the residues, but there was evidence that suppression could occur both during growth, and after cover crop termination. Mechanisms underlying improved efficacy of crucifers compared to cereal cover crops in the suppression of *R. solani* have not only been linked to the ITCs released (Larkin, 2013), but also to the improved soil microbial biodiversity following crucifer cover crops (Ascencion et al., 2015; Cohen et al., 2005;

Wiggins and Kinkel, 2005). This can explain the longer-term effects of cover crops on *R. solani* (Motisi et al., 2009) that last well beyond the period (a few days) in which ITCs remain active in the soil (Gimsing and Kirkegaard 2009). No studies are available on the effect of cover crop mixtures on *R. solani* suppression, but legumes have been reported to be less effective than crucifers in potato systems (Larkin, 2013) and cotton systems (Rothrock et al., 1995). Legumes are host to some strains of *R. solani* (You and Barbetti, 2017; You et al., 2008) so care would be needed if selecting a legume component of a mixture with crucifers for control of that pathogen.

Crucifers are considered to be good break crops for the take-all fungus *G. tritici* in cereal systems. Severity of take-all in wheat has been shown to be entirely controlled when wheat is preceded by canola compared to wheat (29 % severity) as canola does not host the pathogen (Lemerle et al., 1996). But canola was also superior to a fallow (Jadot, 1981), a faba bean (Bowerman and Banfield, 1982) and a linseed break (Kirkegaard et al., 2000) despite these also being non-hosts. This additional suppression by canola was thought to be due to ITC release from canola roots and some studies provided evidence for this in the field (Kirkegaard et al., 1994, 2000). In some cases Indian mustard showed similar or better suppression of take-all than canola (-40 % of disease incidence compared to wheat) and this was thought to relate to the release of 2-propenyl ITC from mustard roots in addition to the 2-phenylethyl ITC released by both species (Angus et al., 1991; Kirkegaard et al., 1997). Overall the effect has been variable (Smith et al., 2004) and highly dependent upon the seasonal conditions which also influence inoculum survival. Legumes can be also considered as break-crops since they are non-host of *G. tritici* (Angus et al., 2015; Cotterill and Sivasithamparam, 1988; Lawes et al., 2013). In comparative studies, lupin showed the same impact as a break crop as canola, and clover and bean had the same efficacy as oilseed rape (Dyke and Slope, 1978). Among the legumes, Speakman et al., (1978) showed that white clover and alfalfa species had the same take-all suppression levels. Thus for a disease such as take-all, the use of crucifer-legume mixtures may provide a valuable disease suppressive capacity while maintaining other benefits of the mixture.

However for some pathogens such as *V. dahliae* it has generally been shown that it is much more difficult to achieve suppression using biofumigation. The pathogen has been found to be less sensitive to the commercial soil fumigant methyl-ITC (known as Metham sodium, Vapam) than other pathogens (Klose et al., 2008) so may be a less suitable target for biofumigant suppression. However high concentrations of dimethyl disulfide and methyl

sulfide appeared to have reduced *V. dahliae* colony counts in bioassay potato stem saps in a glasshouse experiment (Wang et al., 2009). In soil, suppression of *V. dahliae* by a mixture of white and Indian mustards has been observed for potato (50 % of incidence) (Larkin et al., 2011) and for cauliflower with broccoli (-50% of severity) (Xiao et al., 1998). However these same cover crops have not been effective in suppression of *V. dahliae* in tomato (Hartz et al., 2005). ITC-related effects have not been clearly shown in *V. dahliae* suppression, but many studies evoke a different suppressive pathway related to organic matter addition (Berbegal et al., 2008; Ochiai et al., 2008; Olivier et al., 1999a; Subbarao et al., 1999). Indeed in potato fields, Davis et al. (2001) found that the level of organic matter in the soil was inversely related to *Verticillium* wilt severity. Furthermore Michel (2014) showed that mustard hay reduced the number of *V. dahliae* microsclerotia significantly in two greenhouse experiments, but this effect was presumably not caused by crucifer allelochemicals, as the aliphatic GSLs are largely lost during the hay-making process, and because the suppressive effects occurred several months after the hay application when ITCs and other chemicals have dissipated in the soil. Increased soil microbial activity has been suggested as the cause of improved suppression following crucifer amendment (Davis et al., 2010; Larkin et al., 2011; Michel, 2014). Indeed Davis et al., (2010) and Michel (2014) found a negative relationship between *V. dahliae* population (and incidence) and soil microbial activity. The impact of crucifer-legume mixtures on microbial activity and pathogen suppression may be of interest from both an allelochemical and biomass perspective. For example legumes such pea have been shown to suppress *V. dahliae* to the same extent as rape (-40% of incidence) (Davis et al., 2010). However we are not aware of any studies that have investigated the impact of crucifer – legume mixtures on *V. dahliae* populations, nor the suitability of different legume and crucifer species for that purpose.

The effect of biofumigant crucifers on *A. euteiches*, the fungus responsible for pea root rot has been studied extensively, and significant suppression has been demonstrated for Indian and white mustard cover crops (-50% of severity) (Hossain et al., 2015). Mechanisms of suppression have been suggested to be ITC related (Chan and Close, 1987; Muehlcen et al., 1990; Papavizas and Lewis, 1970), but recent studies have also found suppression to be partly due to soil de-compaction (Hossain et al., 2015), generating a less favourable soil environment for *A. euteiches*. For this pathogen, cover crop mixtures of crucifers and legumes would presumably best be avoided. Legumes used as cover crops can host the same strains of *A. euteiches* as the subsequent cash crop legumes, leading to a disservice compared

to bare soil (Levenfors and Fatehi, 2004). Green manures of soybean, snap bean and alfalfa were also ineffective to suppress the pathogen (Williams-Woodward et al., 1997).

Bacterial pathogens have often been found to be less susceptible than fungi to *in vitro* ITC (e.g. Smith and Kirkegaard 2002). Nevertheless a decrease of more than half of the bacterial wilt pathogen *R. solanacearum* abundance and incidence was observed after Indian mustard incorporation on tomato and tobacco crops (Akiew and Trevorrow, 1999; Akiew et al., 1996; Arthy et al., 2002). Olivier et al., (1999b) found a 98% reduction of *R. solanacearum* on tomato crops following turnip rape crops but this effect was not attributed to GSL production. Kirkegaard (2009) has demonstrated both short-term ITC-related and longer-term organic matter-related suppression of bacterial wilt in solanaceous crops, the former more obvious and effective on sandy soils where the ITCs are not adsorbed and inactivated by high clay and organic matter levels in soil. Another bacterial pathogen responsible for common scab on potatoes, *S. scabies,* has also been suppressed by cabbage residues (-90% of incidence) (Gouws and Wehner, 2004). In common with fungal pathogens, few studies have investigated the impacts of crucifer-legume mixtures on pathogenic bacteria.

2.1.2. Pathogen stimulation

Despite the ability of ITCs to suppress *Fusarium spp*. and *Sclerotinia spp*. *in vitro* (Smith and Kirkegaard, 2002), crucifer cover crops host these pathogens, and are currently not considered to be effective to suppress them (Lu et al., 2010).

Incidence of *Fusarium* increased in tomato following incorporation of a mixture of white and Indian mustard (+34%) (Hartz et al., 2005) and following an Indian mustard (+11%) on watermelon (Njoroge et al., 2008). When Indian mustard and cauliflower cover crops were not incorporated in soil, the effects on disease incidence were respectively 3 and 4 times higher on strawberry compared to no cover crop (Lazzeri et al., 2003). Nevertheless incidence of *F. oxysporum* has been shown to decrease after incorporation of canola (-27%), while severity of *F. graminearum* on wheat decreased by 45% and 30 % respectively following canola and Indian mustard crops (without incorporation) (Kirkegaard and Matthiessen, 2004). Concerning *Sclerotinia spp*., Sexton et al., (2007) found a slight increase of incidence (8%) following the use of a mustard cover crop blend, while in some studies a higher microbial diversity after crucifer cover crop use was considered responsible for suppression of the fungus (Duncan et al., 2006; Hao et al., 2003; Ojaghian et al., 2012; Subbarao, 1998).

Mixtures may be an effective option when crucifers promote specific diseases, or are not effective to suppress them. For example, legumes such as hairy vetch can suppress *Fusarium* of watermelon by more than 50% through an increase in the bacterial population in soils (Zhou and Everts 2007). Though no studies exist, mixtures with legumes with this capacity could reduce the disservice of crucifers for this disease if other benefits of the crucifer are compelling.

2.1.3. Impacts on beneficial fungi and bacteria

Beyond pathogen suppressive effects, crucifer biocidal effects (positive or negative) can influence the general microbial populations including beneficial organisms that may impact soil health and fertility. In general, the impact of crucifer cover crops on beneficial organisms has received less attention than pathogens. Here we discuss the current knowledge of crucifer effects on antagonist microbes, and we then consider two specific examples; arbuscular mycorrhizal fungi (AMF) and N-cycling bacteria, with a focus on the potential for legumes in a mixture to mitigate potential disservices associated with sole crucifer cover crops.

2.1.3.1. Impacts on disease suppressive fungi and bacteria

Toxicity of the ITCs can impact non-target organisms in soil (Bending and Lincoln, 2000; Ibekwe et al., 2004; Rumberger and Marschner, 2004) and in the aquatic environment (Schultz et al., 2005). However under field conditions, the effect of crucifer growth and incorporation on the diversity of microbes has been rarely studied. Interestingly, the enhanced competition of copiotrophic soil microorganism on soil-borne pathogens can be favoured by the addition of fresh organic matter in soil (Friberg et al., 2009; Larkin and Honeycutt, 2006). For example after ITC soil disinfestation, saprophytic fungi that can have an antagonistic effect on soil parasitic organisms (e.g. *Penicillium* sp., *Trichoderma*, *Aspergillus* sp., *Gliocladium* sp.) and several others predominate (Welvaert, 1974). These fungi prolong the inhibition of the parasite by having a synergistic action with the ITC called ‘‘induced antagonism’’. Indeed, the most common example is *Trichoderma spp.* which are antagonist fungi for numerous pathogens (Kandula et al., 2015) and have been proven to be less sensitive to ITC than other fungi (Galletti et al., 2008). In soil conditions, crucifers can increase *Trichoderma* as demonstrated by Kirkegaard and Matthiessen, (2004) where both canola and mustard crops (+300%) stimulated *Trichoderma* without residue incorporation (see Kirkegaard et al., 2004). There are no studies on the impact of crucifer-legume mixtures on these soil microbial antagonists. Inclusion of legumes in mixtures may bring services by

improving the functional and specific bacterial diversity as demonstrated when legumes are used at the rotation scale (Lupwayi et al., 1998). Improving spatial and temporal crop diversity at the field scale provides ecological niches for microorganisms and encourages microbial diversity that may improve "induced antagonist" effects. Indeed general microbial population diversity were either unaffected or increased in some mixtures (Nair and Ngouajio, 2012; Reed-jones et al., 2016; Wortman et al., 2013) but the effects can be different, even for species within the same family (Maul et al., 2014; Zhou et al., 2017). Further assessment of commonly used cover crop species of legumes as sole cover crops as well as in mixtures on the populations of disease-suppressive bacteria and fungi is warranted.

2.1.3.2. Impacts on two beneficial organisms

Most crucifer cover crops are non-hosts of arbuscular mycorrhizas fungi (AMF) and may even reduce populations compared to bare soil through ITC suppressive effects. *In vitro*, 2-phenylethyl ITC has been found to be harmful to AMF (Vierhling et al, 2000). Interestingly in field conditions, rocket (*E. sativus*) a crucifer that lacks the GSL precursor Glucosturtiin (2-Phenylethyl-GSL) for 2-phenylethyl-ITC showed no AMF suppression, while Indian and white mustard were suppressive with a 70% and 56% reduction of AMF colonisation respectively on strawberry (Koron et al., 2014). In soil conditions, negative effects of Indian mustard cover crops on mycorrhizal root colonisation of the subsequent maize has been reported compared to vetch cover crops (-43%) (Njeru et al., 2014), and compared to bare soil for rape (-30%) in soybean systems (Valetti et al., 2016). However other studies have shown no negative impact of rape (Pellerin et al., 2007) or radish (White and Weil, 2010) residues on AMF colonisation on maize roots. Inclusion of legumes in cover crop mixtures could be an interesting option to convert the non-host status of the crucifer sole crop to a host status for the cover crop mixture as a whole. Legumes (with the exception of lupins) are reported to increase mycorrhizal fungi abundance and diversity more than other plant families (Duchene et al., 2017) and fungal antagonist communities differed depending on the species of the legume in the crop rotation (Taheri et al., 2016). Legume diversity at the plot level could even enhance AMF abundance as shown for clover species mixtures when soil has been amended with AMF (Zarea et al., 2009). Nevertheless other studies showed that legumes didn't enhance mycorrhiza abundance more than other families (García-gonzález et al., 2018, 2016; Higo et al., 2015). Concerning crucifer-legume mixtures (see Table 6 for details), Wortman et al., (2013) showed that a mixture of vetch and mustard and a mixture of 8 species (including 3 crucifers) did not reduce soil AMF abundance compared to bare soil

but no crucifer controls were present in the study. Lehman et al., (2012) showed that a mixture of canola, vetch and oat had the same number of AMF propagules in soil as the oat sole crop, and had more AMF propagules than the canola sole crop. Finney et al., (2017) found no negative impact of radish on soil AMF abundance in sole cover crop and in mixtures 2 and 9 months after cover crop sowing compared to no cover crop treatments. Mechanisms of AMF-legume root interactions are still to be clarified in crucifer-legume mixtures to better understand the impacts of the inclusion of a non-host AMF crucifer in the rhizosphere on AMF soil colonisation.

In-vitro ITC applications have an impact on key soil bacterial communities involved in critical processes such as nitrogen cycling, including nitrification and nitrogen fixation (Bending and Lincoln, 2000; Rumberger and Marschner, 2004). Indeed, populations of both ammonium-oxidizing and nitrite-oxidizing bacteria and levels of nitrification were reduced by applications of ITC or other volatile sulfur compounds (Bending and Lincoln, 2000; Brown and Morra, 2009; Saad et al., 1996). Nevertheless in field conditions, these effects of crucifers have not been observed. For example nitrifying bacteria were not affected by broccoli incorporation (Omirou et al., 2011) nor by either Indian mustard or white mustard without residue incorporation (Hossain et al., 2015). Furthermore beneficial rhizobacteria (Rhizobium spp.) responsible for nitrogen fixation were not impacted by rape in soybean systems (Valetti et al., 2016) or by mustards in pea systems (Hossain et al., 2015). The same number of pea nodules were found with rapeseed residues compared to a soil left bare (Scott and Knudsen, 1999). In contrast, Muehlcen et al., (1990) found a decrease in pea nodules when rape was incorporated before sowing, as did Hossain et al., (2015) after both white and Indian mustard cultivation without residue incorporation. Nevertheless these effects were attributed more to the better N supply following crucifer green manures than bare soil (causing a lower pea root nodulation) than to a potential biofumigation effect on rhizobacteria. Theoretically, legume inclusion in mixtures should decrease any potential negative effects of the crucifers on soil microbes involved in nitrogen cycling but experiments are needed to confirm this hypothesis.

2.2. Herbicidal effects of crucifer cover crops

2.2.1. Weed suppression

It is widely known that cover crops can reduce weed germination and/or establishment (Haramoto and Gallandt, 2004; Teasdale, 1996). Cover crops mainly suppress weeds

emerging while growing, but can also suppress the weeds infesting the next cash crop (Lou et al., 2016). Weed suppression from cover crops arises mainly from i) competition for abiotic resources (nutrients, light …). (McLenaghen et al., 1996; Teasdale, 1996); ii) allelopathy (Jabran et al., 2015), or iii) induced bacterial suppression (Lou et al., 2016). Levels of cover crop biomass production are often seen as a good indicator of suppressive capacity (Mirsky et al., 2013; Mohler and Teasdale, 1993) but this phenomenon is highly variable since low biomass of some cover crops can also generate high levels of suppression (Björkman et al., 2015). Among cover crops, crucifers are known to be highly effective competitors for abiotic resources thanks to their rapid growth (Brust et al., 2014), but can also generate biocidal effects via production of GSL-related suppressive compounds (Haramoto and Gallandt, 2004).

In vitro application of ITCs has been proven to inhibit or delay weed germination (Brown and Morra, 1997; Kunz et al., 2016) while weed radical growth is also reduced (Bialy et al., 1990; Wolf et al., 1984). However in soil, allelopathy and competition for resources occur simultaneously, and separating these effects can be challenging (Duke, 2015; Falquet et al., 2014; Macías et al., 2014). In addition allelopathy on weeds may occur from roots and from shoot tissues (Asaduzzaman et al., 2014). Though difficult to attribute suppression to specific mechanisms, several studies highlight that crucifer cover crops can be effective to suppress weeds. Some studies highlighted a better weed suppression by crucifers than grasses or legumes (Haramoto and Gallandt, 2005) while others did not (Smith et al., 2014). Some studies including field and controlled experiments concluded that no allelopathic effects (Lawley et al., 2012) were involved in weed suppression, while others have confirmed them (Kunz et al., 2016). Interestingly, mustards have been highlighted as effective suppressers of weeds even at relatively low levels of biomass and/or GSL concentration (Björkman et al., 2015).

The impact of crucifer cover crops on weeds was reviewed by Haramoto and Gallandt, (2004) and more recently by Bangarwa and Norsworthy (2016). They reported weed suppression from crucifer cover crops can be up to 96% compared to bare soil. Weed (bigpod sesbania) germination after incorporation of five different crucifer species decreased by more than 95% (Vaughn and Boydston, 1997). Weed biomass and weed density can be reduced from 85% to 96% after canola compared to bare soil (Bangarwa et al., 2011). Krishnan et al., (1998) showed a 49% reduction of weed biomass after emergence of 3 different crucifer cover crops (Indian mustard, white mustard and rape) with different results for the weed and

cover crop combination. This may relate to different competitive ability or to different allelochemicals released. White mustard cover crop residues reduced the weed infestation by 50 and 60%, and it delayed weed appearance by 3 and 4 weeks for the first and second years, respectively, compared to bare soil (Alcántara et al., 2011). Crucifer cover crops have a negative impact not only on weed density and dry weight but also on weed species diversity compared to fallow treatment (Mennan and Ngouajio, 2012). This study also showed a lower impact of cover crops on perennial weeds compared to annual weeds. They conclude that crucifer cover crops must be coupled with other weed suppression strategies to control both annual and perennial weeds. Some studies reported full weed control when cover crops have been coupled with half of the herbicide dose. Indeed Malik et al. (2008) reported the same weed suppression efficacy as a full herbicide dose when radish incorporation was coupled with half of the herbicide dose in maize systems.

Among the same species, cultivars can also have different suppressive effects as highlighted by Asaduzzaman et al. (2014) with 70 canola species, and by Zubair et al. (2017) with alfalfa. Thus an improvement in weed suppression could be achieved by screening cultivars and selecting those with high weed suppression potential.

Cover crops can also have a longer-term effect on weed populations through microorganism-induced suppression which extends the longevity of a cover crop's effectiveness (Inderjit, 2005; Inderjit et al., 2005; Lou et al., 2016). Indeed Mohler et al. (2012) showed that after pea cover crop incorporation, *F. oxysporum* and *F. chlamydosporum* could have biocide effects on seeds and seedlings with an impact on weed emergence. Microbes can also indirectly impact weeds by activating biocides from water-soluble allelochemicals released soon after cover crop incorporation (Jilani et al., 2008; Williamson et al., 1992).

Mixing cover crop species is one of the strategies available to increase weed suppression compared to sole cover crops. For cash crops, it has been widely shown that improved diversity of plants in agroecosystems can increase weed suppression (Corre-Hellou et al., 2011; Hauggaard-Nielsen et al., 2001). Indeed non-legume/legume mixtures increase light interception and nutrient uptake through niche complementarity for resource use, for example through different shoot and root architecture. Another aspect less considered is the potential for additional allelopathic effects from legume species despite the lower competition for abiotic resources. Indeed many legumes can have allelopathic effects on weeds such as green manures of crimson clover (Dyck and Liebman, 1994), subterranean clover (Nagabhushana et

al., 2001), red clover (Fisk et al., 2001; Ohno et al., 2000), other trifolium species (Liu et al., 2013), hairy vetch (Geddes et al., 2015; Hill et al., 2007; Kamo et al., 2003; Teasdale and Daughtry, 1993) and alfalfa (Chung and Miller, 1995).

Examples of weed suppression by cover crop mixtures often deal with cereal-legume cover crops (Creamer and Bennett, 1997; Hayden et al., 2012) but experiments including crucifer-legume mixtures are scarce (see Table 6 for details). In field conditions Kunz et al., (2016) showed the same level of weed suppression with a cover crop mixture including radish, bristle oat and Egyptian clover as with the radish sole crop. They also showed in controlled conditions that compared to sole cover crops the mixtures had similar weed germination rate, but a longer weed germination time. Furthermore a bispecific mixture including vetch and radish had better weed suppression than either of the sole cover crops (Altieri et al., 2011). In multispecies mixtures where oats had the best weed suppression of all pure cover crops, Smith et al., (2014) showed the same suppression for mixtures as the mustard sole crop while Finney et al. (2016) and Baraibar et al. (2017) showed a better suppression by mixtures than a radish sole crop. No studies deal strictly with crucifer-legume bispecific mixtures for weed suppression.

At the interface between intercropping and cover cropping, some systems involve the intercropping of a rape cash crop with a legume cover crop (also called a companion plant). In these systems, undersown legumes provide better weed suppression (from 20 to 75%) than the sole rape crop due to better soil coverage (Lorin et al., 2015). Living mulch clover sown in between cabbage rows also showed better weed suppression (up to 89%) than sole cabbage late in the season (Brandsæter et al., 1998).

In conclusion, crucifer-legume mixtures show strong potential for weed suppression through high competition and biochemical suppression of weed growth. Furthermore, resilience for weed suppression is increased in mixtures through different species growing requirements and through plant plasticity. Indeed, one species in mixtures can compensate potential deficiencies of other species during the vegetation period to maintain high weed suppression. Total weed suppression is difficult to achieve but the combination of the introduction of cover crops and the strong competition brought by mixtures would potentially make it possible to decrease herbicide use substantially. However the lack of knowledge of mechanisms of suppression makes it difficult to select the best combination of different cover crops. Selection of cover crops mixtures through traits and functional complementarity for

abiotic resource use could be an efficient strategy to improve weed suppression potential of cover crops (Ranaldo et al., 2016).

2.2.2. Herbicidal effects on intercropped or companion legume

Herbicidal effects of crucifers on the companion legume in a cover crop mixture would reduce the effectiveness of the mixture. Crucifers showed to have herbicidal effects on legumes *in vitro* (Brown and Morra, 1997) and in field conditions for legume weeds (Bangarwa and Norsworthy, 2016). Furthermore, crucifers may compete strongly for sulfur (S) and affect legume biological N-fixation. For example Génard et al. (2016; 2017) observed that legumes in a mixture with rape had a lower S content than the sole crop, but did not observe lower N_2 fixation, while Couëdel et al., (2018a) found no strong differences in S status of various legumes when mixed with crucifers. Furthermore Jamont et al. (2013b) found that biological fixation of faba bean was increased when intercropped with rape, even if the shoot N concentration was reduced. Indeed high competition for nitrogen usually stimulates legumes to increase biological N-fixation in mixtures (Jensen, 1996). Interestingly pea fixed more N_2 when mixed with radish than with rye (Andersen et al., 2005) while cowpea had no difference in nitrogenase activity or nodule biomass when mixed with radish or oat (Wortman and Dawson, 2015).

In a study dealing with many crucifer legumes species and cultivars (Couëdel et al., 2018c) did not find any strong incompatibilities for crop development, with the exception of radish and turnip rape which had a stronger competition than other crucifer species by reducing companion legume biomass per plant by 20% and 30% respectively. Furthermore, intercropping of rape cash crops with a legume companion crop did not show any incompatibility of development for the many legumes species tested (Lorin et al., 2015; Verret et al., 2017). Herbicidal effects of legumes can impact crucifer growth as demonstrated *in vitro* with biocidal effects of red clover on wild mustard weeds (Conklin et al., 2002; Ohno et al., 2000). Nevertheless all studies cited above showed that crucifer development improved in mixtures with legumes compared to pure crops except for rocket, that was less competitive for abiotic resources (Couëdel et al., 2018c).

2.2.3. Herbicidal effects on subsequent crops

GSL hydrolysis products such as ITCs generally have a short half-life in soil and don’t persist at detectable levels beyond a few days (Gimsing and Kirkegaard, 2009; Morra and

Kirkegaard, 2002). Only water soluble biocides such as thiocyanates may have a longer herbicidal effects but studies are lacking to test this hypothesis (Brown and Morra, 1996, 1995). As a result, impacts of crucifers on the subsequent crop germination and establishment would presumably be indirect through organic matter incorporation effects such as nutrient cycle impacts or changes in microorganism profiles. Krishnan et al., (1998) found similar soybean yields for many cover crops and a fallow treatment suggesting crucifer residues did not harm soybean plants. As discussed earlier, Scott and Knudsen, (1999) found that peas grown in soil with incorporated rapeseed residues had similar numbers of nodules and rhizobacteria activity to those grown in soil with no residues. Interestingly Al-Khatib et al., (1997) showed that rapeseed residue decreased subsequent pea yield and density by two compared to a rye control while it was not observed following white mustard. Similar to the impact on weeds and companion crops, the impacts of cover crop residues could be due to nutrient availability changes more than allelochemicals as hypothesized for pea nodulation decreases following crucifer cultivation (Hossain et al., 2015; Muehlcen et al., 1990). Furthermore, maize development was reduced by mustard and turnip but not by radishes and canola cover crops (Gieske et al., 2016; Spiassi et al., 2011). Gieske et al. (2016) suggested that nutrient release by crucifer cover crops did not meet the cash crop needs, explaining the negative effects observed. Therefore, optimal cover crop termination date seems key to manage nutrient supply to match subsequent cash crop needs.

Legumes may also produce biocidal effects and may influence subsequent crop development (Mondal et al., 2015), but it is generally accepted that legumes are good preceding crops thanks to nitrogen green manure effects (Couëdel et al., 2018c; Thorup-Kristensen et al., 2003; Tribouillois et al., 2016a). In this way crucifer-legume mixtures have a better nitrogen green manure effect than the pure crucifer, and may improve the growth of the subsequent main crop (Couëdel et al., 2018c). Recycling of other nutrients may also mutualize in mixtures compared to pure crops leading to better nutrition for the subsequent cash crop but studies are still needed to assess the capacity of mixtures to acquire nutrients (Couëdel et al., 2018a; Xue et al., 2016).

In conclusion, herbicidal effects of crucifers do not appear to impact legume development meaning that crucifer-legume mixtures are generally compatible. Furthermore, mixtures reduce weed growth and improve nutrient green manure effects providing improved growing conditions for the subsequent cash crop.

Table 6. Summary of studies dealing with crucifer-legume cover crop mixture effects on living organisms**. "**SC" indicates sole cover crops. Living organisms appear in the same order as discussed in the text. We report a general lack of studies dealing with impacts of crucifer-legume mixtures on fungal and bacterial pathogens.

Living organism	Mixture	Effects of mixtures compared to sole crops and/or bare soil	Period of measurement	References
Arbuscular mycorrhiza fungi (AMF)	White mustard, hairy vetch	No difference in AMF abundance in soil compared to bare soil	32 days after cover crop incorporation	Wortman et al., (2013)
	Canola, hairy vetch, oat,	No difference in AMF propagules in soil compared to oat SC and vetch SC and 600% more than canola SC.	2.5 months after cover crop sowing	(Lehman et al., 2012)
	Canola, hairy vetch	No difference in AMF propagules in soil compared to canola SC and vetch SC	2.5 months after cover crop sowing	(Lehman et al., 2012)
	Radish, red clover, hairy vetch, oat	No differences of AMF abundance compared to all SC and 20% more than bare soil	9 months after cover crop sowing	(D.M. Finney et al., 2017)
	Canola, red clover, hairy vetch, rye	No differences of AMF abundance compared to all SC	9 months after cover crop sowing	(D.M. Finney et al., 2017)
	Radish, canola, red clover, hairy vetch, soybean, sunn hemp, oat, rye	No differences of AMF abundance compared to all SC and 25% more than bare soil	9 months after cover crop sowing	(D.M. Finney et al., 2017)
Weeds	Radish – common vetch, niger, Egyptian clover, oat	Same weed density but 10% longer germination time compared to radish and white mustard sole cover crop.	5 and 9 weeks after cover crop sowing	(Kunz et al., 2016)
	Radish, vetch	77%, 42% and 8% weed biomass reduction compared respectively to bare soil, vetch SC and 8%, radish SC	2 months after cover crop termination	(Altieri et al., 2011)

	Indian mustard, hairy vetch, Sorghum Sudangrass, buckwheat, Rye	No difference of shoot dry weight biomass of ambient weeds compared to all cover crop SC. 33% Less shoot dry weight of surrogate weeds compared to Indian mustard SC.	Weed biomass assessed at the next crop harvest (oat)	(Smith et al., 2014)
	Radish, sunn hemp, soybean, oat	Lower weed biomass than radish SC	3 months after cover crop sowing	(Finney et al., 2016)
	Canola, sunn hemp, soybean, oat	Almost full weed suppression for mixtures as well as for canola SC	3 months after cover crop sowing	(Finney et al., 2016)
	Canola, radish, pea, red clover, cereal rye, oats	Better weed suppression for mixtures compared to all crucifers and legumes sole cover crops.	2.5 months after cover crop sowing	(Baraibar et al., 2017)
Companion legume	Bispecific mixtures with 8 crucifer species and 9 legume species.	No negative development of legumes expected when intercropped with a crucifer (radish and turnip rape reduced legume biomass by 40% per plant).	At cover crop termination	(Couëdel et al., 2018c)
	Rape with service legumes	No negative development of legumes.	During crop growth	(Lorin et al., 2015; Verret et al., 2017)
Nematodes	Radish, vetch	No differences of nematodes per g/soil compared to vetch SC and a non-significant 30% increase compared to radish SC	3 months after sowing	(Barel et al., 2017)
	Radish, vetch	No differences of nematodes per g/soil compared to vetch SC	4 dates from pre-incorporation of cover crops to harvest of the next crop	(Summers et al., 2014)

2.3. Impacts on micro- and macro-fauna

2.3.1. Nematodes

2.3.1.1. Plant parasitic nematodes

ITCs have been proven to have biocidal effects on many nematode families *in vitro* (Ntalli and Caboni, 2017). *M. incognita* (root-knot nematode) is susceptible to 2-phenylethyl, benzyl, 4-methylthiobutyl, and 2-propenyl ITC (Luca Lazzeri et al., 2004; Zasada et al., 2009). Populations of potato cyst nematode decreased due to ITC derived from aliphatic GSL (2-propenyl GSL) and the aromatic GSL gluconasturtiin (2-phenylethyl GSL) as shown for *G. rostochiensis* and *G. pallida* when applying high 2-propenyl levels extracted from crucifer plants (Aires et al., 2009; Lord et al., 2011). A lack of gluconasturtiin in the roots of white cabbage cultivars has been shown to increase population of *Pratylenchus* spp. (root-lesion nematode) (Kabouw et al., 2010). Other field experiments demonstrated that 2-phenylethyl ITC had an impact on *P. neglectus* but clearly demonstrated that other non-GSL mechanisms were also involved (Potter et al., 1998). Furthermore, Vervoort et al. (2014) concluded that changes in nematode communities following Indian mustard soil incorporation were not due to ITC release, but to other mechanisms. Differences in nematode suppression by different crucifer species have been highlighted by some studies but the mechanisms of suppression are not well understood (Fourie et al., 2016; Kruger et al., 2013; Ntalli and Caboni, 2017).

Among mechanisms other than biocidal effects of ITCs, non-host or poor-host status of crucifers are often the key to reducing nematode populations (Kruger et al., 2013; Ratnadass et al., 2012). The selection of a non- or poor-host biofumigant cover crop is the first and most crucial step to control parasitic nematodes and it is highly dependent on the cultivar used (Pattison et al., 2006). Using a biofumigant that hosts nematodes can result in the pest population increasing during the growth stage, irrespective of the biofumigation processes following incorporation and decomposition. Under these circumstances termination of the biofumigant prior to the completion of the nematode life cycle on the biofumigant host is crucial, and is often temperature dependent (Fourie et al., 2016).

The difficult part of choosing an appropriate cover crop is that this host status depends on both the crucifer species, and on the different nematode species present. For example, canola (Mojtahedi et al., 1991) and radish were poor hosts of *M. incognita* (Curto et al., 2005) but some oil radish cultivars were among the best hosts for *M. hapla* (Edwards and Ploeg, 2014).

Indian mustard and turnip were both good hosts to root-knot nematodes (Curto et al., 2005; Edwards and Ploeg, 2014). Rocket (cv. Nemat) has the potential to act as trap crop for the root-knot nematode *M. hapla* (Melakeberhan et al., 2006b), while radishes and white mustard are also trap crops for the beet cyst nematode (*H. schachtii*) by disrupting their sexual differentiation (Müller, 1999; Schlathoelter, 2004). Turnip and rocket must be avoided for use as cover crops for suppression of root-knot nematodes (*M. arenaria, M. incognita* and *M. javanica*) as they are respectively host of 2 and 3 of these species (Liébanas and Castillo, 2004).

Suppressive effects of crucifer cover crops on plant parasitic nematodes are also explained by their impact on the wider antagonist food web through changes in soil microfauna and microflora. Indeed, free-living nematodes appeared to be less susceptible to the toxins produced by crucifer crops than plant-parasitic nematodes (Stirling and Stirling, 2003). Following cover crop incorporation, the abundance of nematode trophic groups changed, and caused an increase in bacterial feeder nematode (Valdes et al., 2012) and non-pathogenic nematode communities (Collins et al., 2006; Gruver et al., 2010; Takeda et al., 2009) which decreased parasitic nematode abundance (Piedra-Buena et al., 2015; Stirling and Eden, 2008; Treonis et al., 2010; Wang et al., 2008, 2006). Furthermore, the negative impact of Indian mustard seed meal amendments was less for beneficial nematode species *Caenorhabditis elegans* than on plant parasitic nematodes (Yu et al., 2007). Nematode suppression has also been linked to an increase in antagonistic bacterial communities following cover crop incorporation (Wang et al., 2008). Interestingly, Riga (2011) showed that it is possible to achieve full control of root-knot (*M. chitwoodi*), lesion nematodes (*P. penetrans*) and cyst root nematodes (*P. allius*) following crucifer green manure in combination with half the recommended rate of pesticide 1,3-dichloropropene (1,3-D, Telone).

Improving plant diversity is seen as a potential solution to improve nematode suppression (Ratnadass et al., 2012) but patterns are not always predictable and the driving mechanisms are poorly understood (Cortois et al., 2017). Indeed, Cortois et al. (2017) showed that for a wide range of grass and legume mixtures the abundance of all nematode feeding types, except for predatory nematodes, increased with both plant species and plant functional group diversity.

Sole legume cover crops can suppress nematodes as shown for hairy indigo and joint vetch on populations of sting nematodes (*B. longicaudatus*) and root-knot nematodes (*M.*

incognita) while velvet bean lowered the populations of several root-knot nematode species (Rhoades and Forbes, 1986; Rodriguez-Kabana et al., 1992). Some tropical legumes (i.e. sunn hemp) also induced a change in nematode communities by increasing the abundance of fungal, bacterial feeding and free living nematodes while decreasing plant-feeding nematodes (Wang et al., 2006). However legumes such as hairy vetch are known to be reproductive hosts of several plant-feeding nematode species (Rich et al., 2009; Timper et al., 2006). Legume cover crops need careful screening to assess the host-non host effect of different species and varieties as demonstrated by Damour et al., (2014) on cover crop legumes in banana cropping systems. Furthermore, McLeod et al., (2001) found that clover was not more susceptible than fodder rape to *M. javanica* but that field pea was more susceptible. Bhan et al. (2010) showed that cover crops that increased nematode numbers when planted as sole crops usually gave the same result when planted in mixtures with another cover crop. For example, mixing a shrub that supressed root-lesion nematode with a susceptible host did not reduce the population of these nematodes (Desaeger and Rao, 2001). In contrast, mixing a legume host shrub of *Meloidogyne* with an antagonistic shrub reduced the populations of this nematode in soil (Desaeger and Rao, 2001). Greater clarification is needed about the host status of mixtures that are composed of host and non-host cover crops.

Interestingly, Cortois et al. (2017) showed that the abundance of nematode plant feeders increased with increasing C:N ratio of the aboveground biomass of the cover crops. In this way, crucifer-legume mixtures may decrease nematode populations compared to crucifer pure crops as their C:N ratio is generally lower (Couëdel et al., 2018c). Nevertheless, very few studies deal with crucifer-legume mixtures and their impacts on specific nematode suppression (see Table 6 for details). A mixture of vetch and radish had the same plant-feeding nematode levels as the vetch sole crop and more than the radish sole crop (Barel et al., 2017; Summers et al., 2014). However, no differences in suppression of potato cyst nematodes have been found when mixing Indian mustard, white mustard and rocket compared to sole crops. Mixtures of white and Indian mustard are in widespread use to suppress plant feeding nematodes, but their efficiency compared to sole crop is not clear (Kokalis-Burelle et al., 2013; Kruger et al., 2013). More purposeful selection of crucifer-legume mixtures combining non-hosting species that may also enhance antagonistic soil populations could be a fruitful area of future research.

2.3.1.2. Impact on non-target beneficial nematodes

Due to different residues and resource use, plant families will not promote the same nematode feeding groups (Orwin et al., 2010; Sohlenius et al., 2011). Indeed, crucifer species have specific effects on nematode communities through the stimulation of different microflora involved in residue decomposition (Collins et al., 2006). For example, radish can enhance bacterial decomposition, while rape and rye increased the fungal decomposition processes (Bhan et al., 2010; Gruver et al., 2010). Bacterial feeding and fungal feeding nematode communities will be influenced by the quality of the residue. Some studies highlighted an increase in beneficial nematode communities (doubling of bacterivores) after crucifer incorporation (Engelbrecht, 2012; Valdes et al., 2012) even though fungivore nematodes tended to decrease (-25%) in some experiments (Valdes et al., 2012). Interestingly biocidal effects of Indian mustard seed meal amendment were on average double forplant parasitic nematodes than for beneficial nematode species *C. elegans* (Yu et al., 2007). Nevertheless, mustards have been shown to have suppressive effects against entomopathogenic nematodes regulating both insect pests or pathogenic nematodes (Henderson et al., 2009; Ramirez et al., 2009). As a result, biocontrol of insects through entomopathogenic nematodes and biofumigation processes could be challenging (Jaffuel et al., 2017).

The effect of crucifer-legume mixtures on general nematode communities have not been widely studied but Cortois et al. (2017) showed that mixing grasses and legumes increased nematode abundance and diversity compared to pure crops. Interestingly the abundance of all nematode feeding types, except for predatory nematodes, increased with both plant species and plant functional group diversity. There appears to be scope for more studies to assess the effects of crucifer-legume mixtures on beneficial nematodes.

2.3.2. Impacts on macrofauna (vertebrate and invertebrate)

Macrofaunal pest suppression is mainly an issue of concern for cover crop development and biomass production itself, as pest damage to the cover crop or the need for protection with pesticides may reduce the effectiveness or compromise the other ecosystem services of the crucifer cover crops. However, crucifer cover crops can also act as a bridge for some pests of the subsequent cash crop and the potential for these disservices must be assessed.

2.3.2.1. Impact on pathogenic insect macrofauna

Crucifers are commonly attacked by many species of insects that are either specialists or generalists (Ahuja et al., 2010). GSL production can act as feeding deterrents for polyphagous herbivores and feeding stimulant for crucifers specialists (Gols et al., 2008; Kuśnierczyk et al., 2007). *In vitro* studies showed toxic effects of ITC (especially aromatic forms) and other GSL breakdown products on many insects such as Diptera, Lepidoptera, Homopetera, Coleopetera and weevils (Björkman et al., 2011; Brown and Morra, 1997). Both levels of GSL and myrosinase concentration can increase in response to herbivore feeding (Hopkins et al., 2009). Interestingly, wild relatives of cultivated crucifers contain more GSL and both generalist and specialist pest insects are less present on them (Gols et al., 2008). High levels of total and individual GSL and myrosinase can even reduce specialist insects such as root flies, moths, aphids, beetles and weevils (Björkman et al., 2011). Indeed, high levels of GSL have been found to have an impact on development of insect's larvae, pupal weight, eggs, body weight and relative growth rate (Björkman et al., 2011). Nevertheless, some studies found no link between GSL profile and suppression of some crucifers specialists (Moyes et al., 2000). A positive relationship has been found between total GSL concentration and herbivore damage, meaning that crucifer resistance is not simply explained by total GSL levels (Giamoustaris and Mithen, 1995). Thus integrated pest management cannot simply rely on GSL-rich crucifers, but must use other methods such as plant mixtures that can generate less favorable conditions for pest reproduction and development.

Risch, (1983) and Andow, (1991) reviewed effects of various plant mixtures on different herbivore pest species and both found that more than half of the pest species were less abundant in mixtures and that around 15-20% were more abundant. More recently, a meta-analysis of Poveda et al. (2008) showed herbivore suppression and natural enemy populations increased in 52-53% of cases while Letourneau et al. (2011) found even higher herbivore suppression (-72%) and antagonist increase (-74%) with the same dataset, but using different analysis methods.

As mentioned above, GSL concentration and types are important considerations in crucifer pest interactions, but these can change in mixtures. Couëdel et al. (2018b) highlighted that GSL concentration remained unchanged in roots but slightly increased in the shoots of crucifer cover crops when mixed with legumes compared to sole crucifer cover crops. Furthermore crucifers in mixtures showed no strong changes in GSL types and proportions. Crucifers in mixtures with legumes could therefore be less susceptible to feeding by

specialists and generalist insect pests on their aerial parts. Nevertheless variability of responses in GSL concentration were observed between crucifer species and among sites, meaning that more studies are needed to understand better the effects of mixtures on GSL profiles. Furthermore these results were different to those of Björkman et al. (2008) who found decreases in aliphatic and indole GSL concentration when cabbage was mixed with clover, and Stavridou et al. (2012) who found a decrease in indolyl GSL in broccoli florets when broccoli was mixed with lettuce in a substitutive crop mixture design.

The main interest of mixtures for macrofauna pest suppression is to i) disrupt the ability of the pest to find a host due to physical barriers of the companion crop and ii) have repellent effects due the odour of the host plant (Boudreau, 2013; Finch and Collier, 2012). For crucifer crops, the effects of companion legumes on pest abundance have mainly been studied for cabbage and broccoli vegetable production (Hooks and Johnson, 2003). As a companion crop, clover species have been widely used and have reduced many pests on crucifer plants (Finch and Collier, 2000). Overall, legume companion crops have a negative effect on i) oviposition, ii) colonization and iii) tenure time (Hooks and Johnson, 2003). Effects on oviposition have been studied by Ryan et al. (1980) who reported fewer eggs laid (-11%) by *D. radicum* on cabbage when intercropped with white clover. More recent studies showed that the cabbage root fly reduced egg-laying by 36–82% when cauliflowers were intercropped with 24 other non-host plant species (Finch et al., 2003). Height of the intercrop species has been shown to impact visual stimuli leading to oviposition of *P. xylostella*, indeed fewer eggs where laid on white cabbage when intercropped with tall compared to short red clover (Åsman et al., 2001). Finch and Kienegger (1997) found that subterranean clover must cover 50% of the vertical profile of cabbage species to reduce eight crucifer pest specialists. These studies confirm results of Weiss et al. (1994) that concluded that a lack of camouflage of canola by low growing pea explained that intercropping has no effect on abundance of *P. cruciferae*. Cadoux et al. (2015) concluded that companion legumes intercropped with rape reduced *C. picitarsis* of rape due to a dilution or visual effect. Visual stimuli can also be affected by overall cover color. Indeed crucifer aphids could be reduced by intercropping as aphids respond to visual stimuli to find host plants by contrasting the plant color with the soil color (Kennedy et al., 1961; Kring, 1972). Other studies showed that visual stimuli for cabbage flies can also decrease due to differences in shape and color of leaves when crucifers where intercropped (Altieri and Liebman, 1986; Finch and Collier, 2000; Langer et al., 2007).

Mixtures can be ineffective against some insects when there is a strong ability to find the host such as for *P. rapae* (Reddy, 2017). Interestingly, populations of *P. rapae* and *P. xylostella* were even found to be higher on broccoli mixed with a high diversity of nectar producing plants (from 34 genera) than for the broccoli sole crop (Zhao et al., 1992). In such cases, it is crucial that nectar production of the companion plant doesn't attract further pest insects of the crucifer (Zhao et al., 1992). It is also important that the companion crop doesn't attract pests of the planned subsequent cash crop. For example *A. pisum* can be increased by crimson clover and hairy vetch cover crops (Kaakeh and Dutcher, 1993).

Beyond physical effects, companion plants also combine chemical barriers for insects as their tenure time is reduced in mixed stands as the presence of a complex sensory environment may distract pests from settled feeding (Bernays, 1999). Tenure time of *P. cruciferae* was longer in pure broccoli than broccoli intercropped with faba bean and common vetch (Garcia and Altieri, 1992). These results were confirmed by Elmstrom et al., (1988) who found that *P. cruciferae* had a faster rate of immigration and spent less time on broccoli in mixtures with white clover than in pure broccoli crops.

Mixtures of different crucifer species can also decrease insect abundance. For example, flea beetles (Altieri and Gliessman, 1983; Andow et al., 1986) and aphids (Costello and Altieri, 1995; Kloen and Altieri, 1990) had lower populations in crucifer-crucifer mixtures. Mixed crucifers can also lead to trap crop systems where one of the crucifers aimed to attract the insect to leave the other crucifer free of attacks. Indian mustard for example, is highly attractive to many insects of cabbage (Hooks and Johnson, 2003). Crucifers can even act as a dead-end as for the weed wintercress (yellow rocket) which was highly attractive for oviposition by female *P. xylostella*, but the larvae could not survive on the plant (Badenes-Perez et al., 2004). A fascinating area of work in relation to insect interactions involves the work to capture the benefits of the ancient intercropping of the GSL-containing mashua with potato in the Andes, in which suppression by the mashua companion plant provides protection from potato weevils (*C. formicarius*) and late blight (*Phytophthora infestans*) (Ortega et al., 2006). Attempts have been made to engineer the benzyl-GSL biosynthesis pathway into the potato leaves to provide *in planta* protection in contemporary potato monocultures (Geu-Flores et al., 2009).

In conclusion, despite several studies dealing with insect suppression by crucifer-legume cover crop mixtures, impacts on several specific crucifer pests have scarcely been studied

including cabbage-stem flea beetle (*Psylliodes chrysocephala* and *Phyllotreta nemorum*) or even pollen beetles (*Meligethes aeneus* and *Meligethes viridescens*). Screening the suppressive effects of crucifers and legumes on insect pests along with interactive effects is required to avoid inadvertent disservices resulting from inappropriate mixtures that may exacerbate insect damage within the system.

2.3.2.2. Impact on slugs

Slugs are considered as one of the main concerns for cover crop use due to the potential to act as a bridge for slugs that infest the next cash crop. It is especially a concern for slug-sensitive cash crops such as sunflower, wheat or triticale.

Even if molluscs can be found under crucifer plants (Glen et al., 1990) it has often been found that they avoid plants with high GSL concentration (Barone and Frank, 1999; Byrnes et al., 2014). Nevertheless concentrations of GSL in agriculturally important crucifers are generally lower than wild types and can attract slugs (Stowe, 1998). Field data are lacking on other compounds that can be deterrents for slugs such as terpenes or tannins (Albrectsen et al., 2004; Fritz et al., 2001). Interestingly grey field slug (*Deroceras reticulatum* Muller) damage has been found to be inversely proportioned to total GSL concentration in rape (Glen et al., 1990) as also confirmed by Giamoustaris and Mithen (1995) for other slugs. Interestingly, it was specifically the increase in 3-indolyl methyl-GSL (glucobrassicin) and 2-phenylethyl-GSL (gluconasturtiin) that were responsible, even when the total GSL concentration decreased.

Reduced sensitivity to slugs was found for black mustard and *Arabidopsis* when mollusc mucus had been previously applied, due to the activation of the jasmonic pathway, and a higher production of GSL (Falk et al., 2014; Orrock, 2013). Slug damage on plants also attracts natural enemies of slugs through the emission of volatiles via the jasmonic pathway (Walling, 2000). Some studies have shown that crucifer cover crops such as mustard compared to bare soil, can either have no effect or decrease slug abundance prior to potato planting (Frost et al., 2002; Silgram et al., 2015).

Slug palatability can vary significantly between cover crops species (Briner and Frank, 1998; Charles et al., 2012; Le Gall and Tooker, 2017). Palatability of crucifers are generally high for rape, but low for some other crucifer cover crops such as Indian mustard and radish (Robin, 2011). Palatability of legumes are moderate for some cultivated species such as forage pea and crimson clover and low for cover crops such as faba bean and common

vetches (Robin, 2011). Compared to other legumes tested, alfalfa had lower slug population growth (Anon, 2002). Slug damage has been found to double after red clover or vetch compared to ryegrass indicating that outside palatability, microclimate and foliage type may also play a role in slug abundance (Vernavá et al., 2004).

Surprisingly given the significant issue with slugs, we found no studies dealing with slug abundance in crucifer-legume cover-crop mixtures, although the studies discussed above suggest interactions are likely to arise between slugs and diversified cover crops. It would appear cover crop screening and mixture interactions could be a useful area of research to seek opportunities to reduce the overwintering of slugs and reduce pesticide use.

2.3.2.3. Impact on beneficial macrofauna

ITCs may attract parasitoids of crucifers pests; for example parasitoids of *D. brassicae* were attracted by traps baited with 2-phenylethyl ITC (*Platygaster subuliformis*) or 2-propenyl ITC *(Omphale clypealis)* (Murchie et al., 1997). The parasitic wasp (*Diaeretiella rapae)* of a cabbage aphid (*Brevicoryne brassicae*) is attracted by 3-butenyl ITC (Bradburne and Mithen, 2000). However, ITCs can also act as repellants to beneficial macrofauna. For example in laboratory experiments, ITCs influenced decomposer organisms such as Collembola *(Folsomia fimetari)* (Jensen et al., 2010), and the beneficial soil arthropod *Folsomia candida* had reduced survival and reproduction when pure 2-phenylethyl ITC at low concentration was applied into the soil (Van Ommen Kloeke et al., 2012).

Overall, cover crops and mulch at the soil surface generally enhance earthworm abundance (Bautista-Zúñiga et al., 2008; Ortiz-Ceballos et al., 2007). In the field, Kabouw et al. (2010) found that a high degree of intraspecific variation in root GSL profiles had no impact on earthworms and Collembola abundance. Furthermore, Fouché et al. (2016) found that broccoli, mustard and oilseed radish had no significant effect on earthworm (*Eisenia andrei*) survival or growth, even if broccoli reduced earthworm reproduction by 37%. Nevertheless, Zuluaga et al. (2015) found that high GSL cabbage cultivars could have toxic effects on springtails and earthworms, and in particular they related this to high levels in aliphatic GSLs.

As for insects pests, the use of mixtures could also impact the abundance of beneficial natural enemies through different barriers (e.g., physical, olfactory). In a general review of various intercropped plants and various beneficial species, Andow (1991) found that the population of natural enemies of pests was higher in the intercrop or species mixtures in 53% of the

studies, and lower in 9%. Indeed the main hypothesis was that if parasitoids have a strong association with their insect host and/or with their host plant, this will not be affected in a mixture (Andow, 1991). However, some studies suggested that predators may be more sensitive to habitat type than to prey density (Hooks and Johnson, 2003).

It is therefore necessary to study the impact of species mixtures on both the pests and antagonists at the same time in order to identify conditions where antagonists are enhanced at the expense of pests, and to avoid the risk of suppressing antagonists without impacts on their pests. For example cabbage intercropped with white clover reduced the number of *D. radicum* pupae per plant but also the probability that the pupal parasitoid (*Aleochara bilineata*) located in plants infested with *D. radicum*. Reductions in overwintering *D. radicum* could then be mitigated by a decrease in pupal parasitism in this system (Brown and Anderson, 1999). Similarly, compared to broccoli sole crops, Costello and Altieri (1995) found a lower incidence of the parasitoid Braconidé *Diaretiella rapae* on aphids (*Myzus persicae* and *Brevicoryne brassicae*) for a few different species of clovers and trefoil intercropped with broccoli. More antagonists of aphids (*Brevicoryne brassicae*) were found when broccoli was intercropped with mustard, and this resulted in lower aphid infestation (-40%) (Ponti et al., 2007). Interestingly, faba bean intercropped with rape was shown to attract an aphid antagonist (*Diaeretiella rapae*) due to nectar production (Jamont et al., 2014; 2013a).

Plant diversity through broader diets could also be useful to keep a level of specialist predators that may face periods of inadequate prey availability (Limburg and Rosenheim, 2001). As for microbial communities, high organic matter accumulation from diversified biomass can increase diversity of macrofauna such as decomposers (Brown and Oliveira, 2004). Communities of worms seem favoured by legumes (Ortiz-Ceballos et al., 2007). Crucifer-legume mixtures could be interesting to avoid potential negative effects of crucifers on worms as highlighted above. Cover crop legumes as sole crops are especially interesting to increase carabid activity-density (O'Rourke et al., 2008), but can also have similar effects when intercropped with wheat compared to wheat alone (Carmona and Landis, 1999). Alfalfa and kura clover intercropped either with maize and soybean can also increase carabid abundance (Prasifka et al., 2006).

A higher diversity of cover crop plants could also attract natural enemies through nectar and pollen sources for predatory insects such as shown with the use of crimson clover when

grown with cotton (Tillman et al., 2004). Indeed diversified covers can enhance higher abundance and diversity of pollinators through complementarity in flowering properties such as flower nutritional value and blooming date resulting in a longer flowering period (Bretagnolle and Gaba, 2015; Potts et al., 2010; Pywell et al., 2005). Differences among the effectiveness of crucifer species to attract pollinator insects has been previously highlighted (Eberle et al., 2015). For example, *Camelina* spp. showed higher pollinator visits than pennycress but intercrops of both species may bring higher diversity of pollinator (Groeneveld and Klein, 2014). Forage legumes have been shown to be especially interesting for bumblebees, but there is a lack of information on other non-dominant groups of pollinator (other than *Apidea*), and very few data exist for other legume cover crops (clovers, vetches, lupin etc). Nevertheless, Ellis and Barbercheck, (2015) reported that flower density was a key factor influencing the frequency of bee visitation and that this density can logically decrease in mixtures due to "dilution phenomenon". They showed that canola attracted more bees than both pea and red clover, and that canola cropped alone attracted more bees than when mixed with 3 or 5 other species including legumes, radish and rye. Potts et al. (2010) confirmed these results by showing that plant diversity *per se* did not increase bumblebee abundance, but species mixtures that increased flowering plant density did. Ellis and Barbercheck (2015) also reported that these different cover crops attracted unique bee communities through different blooming phenology and nutritional values that may increase the diversity of pollinator communities.

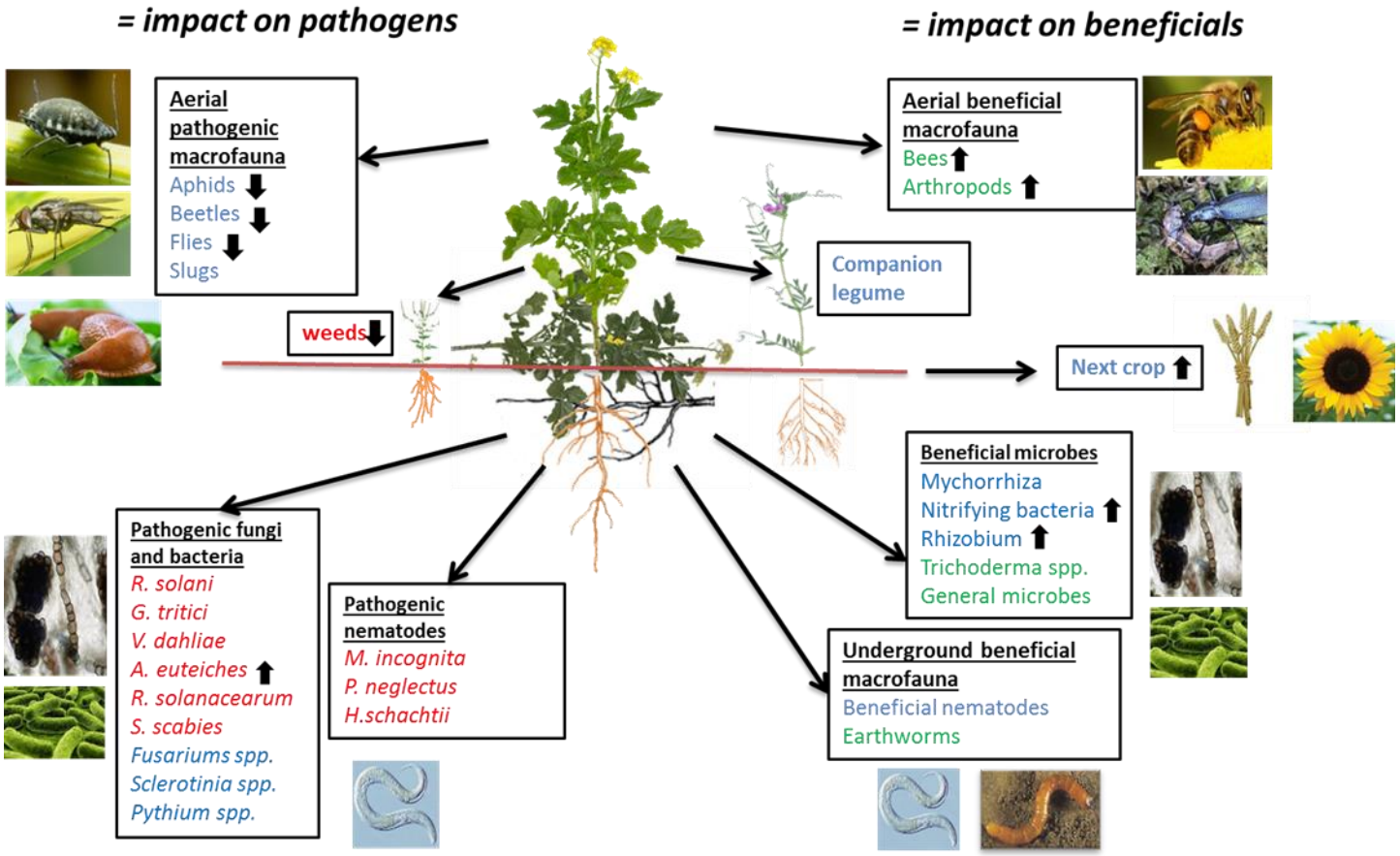


Figure 7. Examples of services and disservices provided by cover crops on a wide range of pathogens and beneficial organisms. Crucifer sole crops may favor (green text), suppress (red text) or have neutral/less well understood effects (blue text) on living organisms. Crucifer-legume mixtures may favor (⬆), suppress (⬇) or have unknown effects (no arrow) compared to crucifer sole crops.

2.4. Interactions and trade-offs for indirect biotic suppression

As shown on Figure 8, many links exist between different organisms that could increase the impact of cover crops on pathogens, and these interactions are considered in this section.

Beneficial macrofauna can play a key role in pest regulation as they have an impact on weeds, pathogenic fungi and pathogenic nematodes. For example, ground beetles (Coleoptera: *Carabidae*) act as antagonists of weed seeds, other insects and slugs (Bohan et al., 2011). High levels of earthworms coupled with trefoil cover crops decrease pathogenic nematodes (Boyer et al., 1999). Effects of earthworms could be mediated by i) macropore creation that could impair nematode movements and ii) direct feeding on nematodes (Blanchart et al., 1999; Boyer et al., 2013). High acarian density following mulch was also found to reduce abundance of plant parasitic nematodes (Badejo et al., 2002; Peachey et al., 2002). Weed seed predators such as carabid are enhanced by cover crops (Gallandt et al., 2005; Ward et al., 2011) and high levels of weed predation have been reported by Heggenstaller et al. (2006) and Puricelli et al. (2005) in different cropping systems. Complementarity for weed predation has also been found between vertebrate and invertebrate macrofauna (Harrison et al., 2003). Furthermore, Ang et al. (1995) observed a competition synergy on weed suppression (Creeping thistle) between cover crops (tall fescue and crownvetch) and the weed seed predator *Cassida rubiginosa*. The suppression of weeds is important as they can act as a biological bridge to enhance plant parasitic nematodes and arthropod pests through host effects (Norris and Kogan 2005, Creech et al. 2007, Groves et al. 2001). Cover crop choice becomes complex as cover crops resistant to plant parasitic nematodes are not always the best to suppress weeds (Damour et al., 2014).

Otherwise, entomopathogenic nematodes play an interesting role to suppress pest insects. Nevertheless mustard cover crops used to suppress plant parasitic nematodes had a negative effect on beneficial entomopathogenic nematodes (*Steinernema* spp. and *Heterorhabditis* spp.) and decreased associated insect pest control (Henderson et al., 2009; Ramirez et al., 2009).

Insect pathogenic fungi such as *Hypocreales* spp. are known to kill pathogenic nematodes (Klingen et al., 2002b; Meadow et al., 2000). Those within the order of *Entomophthorales* are antagonist of *D. radicum* and *D. floralis* (Klingen, 2000; Klingen et al., 2000). Interestingly, ITC released by rocket was found to be toxic to germination and growth of insect pathogenic fungi of *Galleria mellonella in vitro*, while no inhibition on *Tolypocladium cylindrosporum*

and an enhancement of *Metarhizium anisopliae* (*M. anisopliae*) insect pathogenic fungi was observed in soil in microcosms (Klingen et al. 2002a). Soluble extracts of other crucifer species can enhance germination and development of *M. anisopliae* on the mustard beetle (*Phaedon cochleariae*) (Inyang et al., 1999). Another study showed that cover crops and other practices enhancing soil biological diversity enhanced insect pathogenic fungi *M. anisopliae* and *Adelina* sp. and increased suppression of larvae population of the Coleoptera *Antitrogus parvulus* in sugar cane crops (Allsopp et al., 2003).

These interactions and biological regulation between living organisms represent an interspersing of indirect biocontrol effects of cover crops, especially when beneficial macrofauna and insect pathogenic fungi can be enhanced.

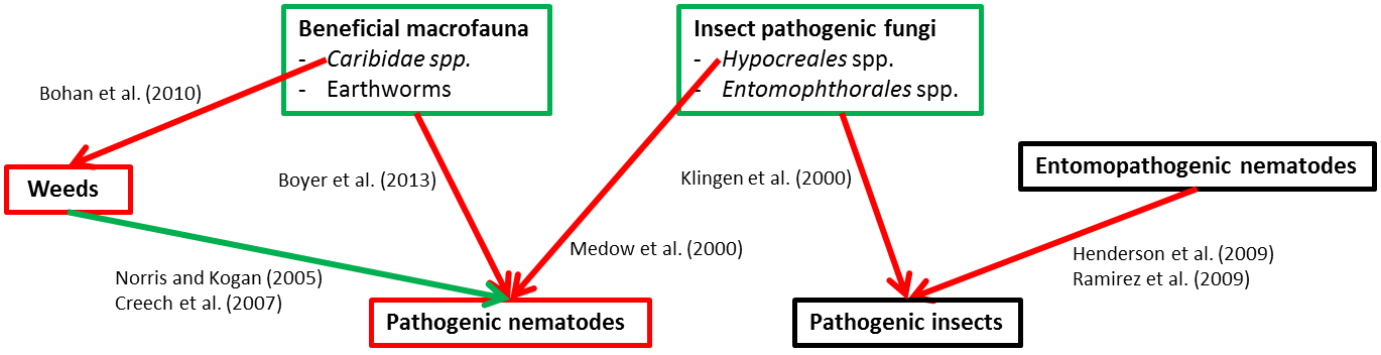


Figure 8. Specific examples of biotic interaction between living organisms providing indirect pathogen suppression from pure crucifer cover crop use. Green, red and black frames respectively represent positive, negative and neutral (or poorly understood) effects of crucifer sole cover crop on living organisms. Green and red arrows represent positive and negative effects respectively occurring between living organisms and relevant literature are provided.

2.5. Summary of research opportunities to support a new multiservice paradigm

Our review of the pest and disease suppression potential by crucifer sole crops reinforces the widely reported pest-suppression potential they exhibit, although the suppressive potential is clearly quite variable for different target organisms. Disservices generated by negative impacts on beneficial organisms were scarce and may not represent a strong concern when selecting a crucifer cover crop for biocontrol. Often there was a focus on individual crucifer species for suppression of a given pathogen, and there may be potential to consider a more diverse range of crucifer species. In that context, definitive links between pathogen suppression and GSL-derived allelochemicals, previously emphasized by Matthiessen and Kirkegaard (2006), were still missing from many studies. Such links are important to make informed selections of specific crucifers rich in biocidal GSLs where this forms a critical service for the cover crop. However, disease suppression was not always associated with higher GSL-producing crops, and was also observed with non-crucifer crops to a similar degree, indicating that other mechanisms and interactions are important. In that sense, sole crucifer cover crops may not always be the best solution to suppress pathogens. The level of added organic matter needed for pathogen suppression may be a more important driver of pest suppression, and then it becomes important to understand the relative merits of sole crops and mixtures. It can be a difficult methodological task to separate experimentally these effects, as the amount of organic matter added is also linked to allelochemical production in most cases. Experiments including appropriate control treatments without biocidal molecules are needed to separate these effects (discussed further in Section 3).

The summaries in Table 6 and Figure 7 emphasise the overall lack of experiments dealing with the suppressive effects of cover crop mixtures on soil-borne fungal and bacterial pathogens. Above-ground organisms such as insects and weeds have been the focus of several studies on cover-crop mixtures which may relate to the relative ease with which they can be monitored compared with below-ground pathogens such as fungi and bacteria. Thus the impact of mixtures on a wide range of belowground organisms, both pathogenic and beneficial, remains to be investigated. For crucifer-legume mixtures, the main concern in terms of pathogen management is to select the best species mixtures for suppressive effects that also avoid pest enhancement (through hosting). Screening both crucifer and legume species of interest for host status of the most relevant pests and pathogens is a first crucial step. Screening at the cultivar scale may also be a necessary refinement as host, non-host and allelopathic potential have all been shown to be potentially cultivar dependant (Asaduzzaman

et al., 2013; Damour et al., 2014). Beyond the host status effects, below-ground interactions in species mixtures for key beneficial organisms such as AMF and rhizobia are also of interest to avoid the potential nutrient disservices of the cover crop mixtures. Recent advances in molecular techniques applied to the ecology of soil-borne organisms provide a major advance to help unravel below-ground interactions in mixtures (Canfora et al., 2016; Orgiazzi et al., 2015; Yang et al., 2014). These new tools overcome some of the difficulties in measuring and monitoring the populations and dynamics of fungal and bacterial pathogens and provide significant scope to shed light on the potential for multi-services provision of crucifer-legume mixtures.

Chapter 3. Cover crop crucifer-legume mixtures provide effective nitrate catch crop and nitrogen green manure ecosystem services.

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Graphical representation of research objectives:

In this chapter for various cultivars of nine crucifer and height legume species we ask the questions:

- Do mixtures reach the same nitrate catch crop effects than pure crucifers?
- In which extent do mixtures have a better green manure effect than pure crucifers?
- Are we able to highlight combination of species that are either highly compatible or incompatible to mutualize services linked to N cycle?

	Crucifer sole crops	Legumes sole crops	Crucifer + Legume
Catch Crop service	**Strong effect** Leaching reduced up to **75 %** compared to bare soil (Justes et al. 2012)	**Moderate effect** Leaching reduced up to **60 %** compared to bare soil (Tribouillois et al. 2016)	?
Green manure	**Low effect** Potential net organisation of residus (Justes et al. 1999)	**Strong effect** Net mineralisation of residus (Thorup-Kristensen et al. 2003)	?

Figure 9. Summarized scheme of questions raised by the chapter 3

1. Introduction

Intensive agricultural practices and bare soils during fallow periods can cause nitrate pollution of ground water. Cover crops grown during the autumn-winter period between two cash crops in annual rotation are an effective solution to decrease nitrate leaching (nitrate pollution mitigation service) due to their ability to capture soil mineral nitrogen (SMN) (Meisinger et al. 1991; Constantin et al., 2011). Cover crops can also provide a green manure service by releasing acquired nitrogen (N) to the subsequent cash crop after incorporation into the soil (Kramberger et al., 2009; Justes et al., 2012). The green manure service has been found to be inversely proportional to the C:N ratio of cover crops (Quemada and Cabrera, 1995; Justes et al., 2009). Compared to bare soil, non-legume species grown as catch crops effectively decrease nitrate leaching, while leguminous species or legumes are the most efficient to ensure N green manure (Thorup-Kristensen et al., 2003). Non-legume species can capture up to 70% of SMN during the fallow period (Justes et al., 2012). Among them, Brassicaceae species or crucifers such as turnip rape (*Brassica rapa*), white mustard (*Sinapis alba*) and fodder radish (*Raphanus sativus*) are often considered the most effective catch crops due to their rapid increase in root depth and density (Thorup-Kristensen, 2001; Thorup-Kristensen et al., 2003), which allows them to capture a large amount of N soon after sowing, decreasing nitrate leaching and water pollution (Thomsen and Hansen, 2014). Crucifers provide less green manure service than legumes due to their moderate C:N ratio (range = 15-25), which induces slower mineralisation of the cover crop residues after their incorporation into the soil (Justes et al., 2009). Legumes usually provide the most effective green manure service due to their low C:N ratio (range = 10-15), which induces faster mineralisation, and of a larger proportion, of their acquired N present in their residues returning to the soil (Tonitto et al., 2006). Some of the N that legumes acquire comes from atmospheric N_2 fixation, meaning that exogenous N is added to the agroecosystem (Peoples et al., 1995). Certain legumes can also provide a catch crop service, although not as much as crucifers, and can decrease SMN during the fallow period by up to 60% compared to that under bare soil, depending on the legume species and site (Tonitto et al., 2006; Tribouillois et al., 2016a; Wortman, 2016).

Cover-crop mixtures composed of legume and non-legume species could provide both catch crop and green manure services simultaneously by combining advantages of both sole crop species (Ranells and Wagger, 1997; Kramberger et al., 2009; Tosti et al., 2014). The green manure service of cover crop mixtures generally lies between those of non-legume sole crops

and legume sole crops because mixtures have lower C:N ratios and acquire more N than non-legume sole crops (Kuo and Sainju, 1998; Tosti et al., 2012 Kramberger et al., 2013). Mixtures also provide a catch crop service similar to that of non-legume sole crops (Möller et al., 2008; Tosti et al., 2014; Tribouillois et al., 2016a). It has been shown that three months after sowing, a mixture with turnip rape and white mustard provided more catch crop service than one with the non-legumes foxtail millet (*Setaria italica*), bristle oat (*Avena strigosa*), Italian ryegrass (*Lolium multiflorum*) or phacelia (*Phacelia tanacetifolia*), and maintained a good green manure service (Tribouillois, 2014), indicating that crucifers are good candidates for providing efficient nitrate catch crop effect in mixtures.

Mixtures also increase N-related ecosystem services because of their high resource use efficiency, due to niche complementarity in using abiotic resources such as light, water and nutrients (Jensen, 1996). Overall, compared to crucifer sole crops higher N acquisition in crucifer-legume mixtures has been demonstrated for grain production (Andersen et al., 2005) and cover crops (Tribouillois et al., 2016; Wendling et al., 2017). However, legumes grown in mixtures may suffer from the competition engendered by crucifers (Szumigalski and Van Acker, 2008; Wortman et al., 2012; Tribouillois et al., 2016). Crucifers could be a poor companion crop for two main reasons: 1) they strongly compete for water, nutrients and light due to their rapid root and shoot growth and 2) they can have an allelopathic effect on legumes due to their production of glucosinolates, which are exuded by roots and transformed in the soil into biocides such as isothyocianates (Chew, 1988b; Matthiessen and Kirkegaard, 2006). Competition for abiotic resources varies greatly among crucifer species. For example, species and cultivars in the crucifer family have differences in shoot and root architecture and plant ontogeny that can induce different interactions with the intercropped legume. Crucifers' production of glusosinolates can vary greatly in the types of molecules and their concentrations (Kirkegaard and Sarwar, 1998) which generate potential and actual allelopathic effects on the legume. As a consequence, the balance between compatibility and incompatibility between crucifer and legume species in mixtures must be studied to determine the most effective bispecific mixtures.

From a practical viewpoint of cover cropping, few experiments have been published that consider root biomass and N content of crucifer-legume mixtures. Roots can contain a high proportion of the N of certain crucifer species, such as fodder radish and turnip, because their taproots have different C:N ratios than their shoots (e.g. Thorup-Kristensen et al., 2003). This indicates that roots must be considered to accurately predict the N green manure service

generated by the N released by mineralisation of cover crop residues incorporated into the soil, which determines N availability for the subsequent cash crop.

The aim of our study was to analyse crucifer-legume mixtures -or Brassicacea-Leguminous mixtures- composed of a wide range of species and cultivars. Our study assessed two key aspects of cover crops: 1) levels of green manure and catch crop ecosystem services provided as sole crops and in mixtures and 2) the type of interaction between the two species (complementarity or competition) involved in N acquisition. Three hypotheses were tested:

1) Mixtures have higher N acquisition, a lower C:N ratio, and more green manure service than crucifer sole crops.

2) Mixtures provide more catch crop service than legume sole crops and almost the same level of service than crucifer sole crops, and then provide multi-ecosystem services.

3) The most suitable compromise between catch crop and green manure services depends on interspecific interactions that maximise the complementarity of both species.

Recent studies have highlighted that climate, soil type and N availability at sowing have the greatest influence on mixtures performances (Tribouillois et al., 2016a). Accordingly, we assessed performances of crucifer-legume mixtures by conducting field experiments from late summer to late autumn for two years at two sites with different soil and climate conditions, which allowed us to assess the generality of species-mixture functioning.

2. Materials and Methods

2.1. Experimental design and cover crop management

Four field experiments were conducted in two years (2014 and 2015) at two sites: 1) the Lamothe experimental farm of INP-EI Purpan, located in Seysses, 20 km south of Toulouse, south-western France (43.506° N, 1.237° E), and 2) the La Vannelière research station of Jouffray Drillaud, located 50 km south-east of Orléans, central France (47,776° N, 2.098° E). Experiments conducted in 2014 and 2015 are referred to as L2014 and L2015, respectively, at Lamothe (L) and as V2014 and V2015, respectively, at La Vannelière (V). According to the Köppen climate classification, Lamothe (Toulouse) and La Vannelière (Orléans) have an oceanic climate (temperate without dry season and with warm summers) (Table 7). At all four site-years, the experiment was a completely randomised design that was replicated with three replicates in blocks. Surface area of the elementary plot, containing 10 rows for each

treatment, was 18.0 m^2 for L2014 and V2014, 22.5 m^2 for L2015 and 20.0 m^2 for V2015. To avoid plant-plant competition effects between adjacent treatments, only the six rows in the middle of the plot were harvested and used for soil measurements.

Table 7. Management, soil and climate conditions of the four experimental sites. Climate data are sums or means of growing season measurements from the sowing date to the date of cover crop termination (biomass harvest date). L2014 and L2015 are experiments at Lamothe in 2014 and 2015, respectively. V2014 and V2015 are experiments at La Vannelière in 2014 and 2015, respectively.

Characteristic	L2014	V2014	L2015	V2015
Sowing date	19 August	31 August	24 August	22 August
Biomass harvest date	4 November	18 November	3 November	26 October
Preceding crop	Fallow (3 years)	Winter barley	Winter wheat	Winter barley
Soil texture	Silt clay loam	Loam	Silty clay loam	Sandy loam
SMN at sowing (kg N ha^{-1})[a]	93	139	48	79
Cumulative irrigation (mm)	30	16	15	0
Cumulative rainfall (mm)	140	250	90	174
Daily mean temperature (°C)	19.0	14.0	18.2	12.4

[a] Soil mineral nitrogen (SMN) measured at a depth of 0–90 cm for L2014 and L2015 and 0–60 cm for V2014 and V2015.

Cover crop species were selected for their ability to grow rapidly during the autumn in a short-term fallow period. Crucifer and legume species and cultivars were selected for their diversity in shoot/root architecture and precocity. All mixtures (Table 8) contained one crucifer and one legume (bispecific mixtures) and were designed to minimise competition according to expert knowledge and recently published information (Tribouillois et al., 2016a). The following species were used: 1) the crucifers or Brassicacea species of rape (*Brassica napus*), white mustard (*Sinapis alba*), Indian mustard (*Brassica juncea*), Ethiopian mustard (*Brassica carinata*), turnip (*Brassica rapa* subsp. *rapa*), turnip rape (*Brassica rapa* subsp. *oleifera*) , radish (*Raphanus sativus*) and rocket (*Eruca sativa*) and 2) the legumes or Leguminous species of Egyptian clover (*Trifolium alexandrium*), crimson clover (*Trifolium incarnatum*), common vetch (*Vicia sativa),* purple vetch (*Vicia benghalensis*), hairy vetch (*Vicia villosa),* pea (*Pisum sativum*), soya bean (*Glycine max*), faba bean (*Vicia faba*), and white lupin (*Lupinus angustifolius*).

Table 8. Experimental design for the four site-years of this study. Letters in cells means that cultivars of crucifers and legumes were mixed in Lamothe in 2014 (a), la Vannelière in 2014 (b), Lamothe in 2015 (c) and la Vannelière in 2015 (d). For all mixtures, the corresponding sole crops were also sown. Empty cells ("/") indicate that the cultivar was not sown in mixtures or as sole crops.

Species		Egyptian clover	Purple vetch	Common vetch			Hairy Vetch		Crimson clover	Soya bean	Faba bean	Pea	Lupin
	Cultivars	*Tabor*	*Titane*	*Spido*	*Nacre*	*Ardente*	*Massa*	*Savane*	*Cegalo*	*Merlin*	*Divine*	*Ascension*	*Arabella*
Rape	*Mosa*	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	/	b
	Ringo	c	c	c	/	a,b	/	/	a,b	/	/	/	/
White mustard	*Abraham*	c	c	c	a,b	/	/	/	/	/	/	/	/
	Architect	c,d	c,d	c,d	a,c	/	c	/	/	/	/	c	/
Indian mustard	*Brons*	c	c	c	/	/	/	/	/	/	/	/	/
	Etamine	c	c	c	/	/	/	/	/	/	/	/	/
Ethiopian mustard	*Capucino*	c	c	c	/	/	/	/	/	/	/	/	/
	Carbon	c	c	a,b,c	/	/	a,b	/	/	a,b	/	/	/
	Utopia	/	/	a	/	/	a	/	/	a	/	/	/
Turnip	*Samson*	c	c	c	/	/	/	/	/	/	/	/	/
Turnip rape	*Chicon*	c,d	a,b,c,d	c,d	/	/	/	/	/	/	a,b	/	/
	Hector	/	a	/	/	/	/	/	/	/	a	/	/
Radish	*Arena*	a	/	/	/	/	/	a	/	/	/	/	/
	Nemaflex	c	c	c	/	/	/	/	/	/	/	/	/
	Terranova	a,b,c,d	c,d	c,d	/	/	/	a,b,c	/	/	/	/	b
Rocket	*Trio*	c	c	c	/	/	/	/	c	/	/	/	/

Species grown as sole crops were sown at densities recommended by cover crop seed companies, breeders (RAGT and Jouffray-Drillaud) and agricultural advisors. Densities in sole crops were 800 plants/m^2 for all clover species, 150 plants/m^2 for Ethiopian mustard, 100 plants/m^2 for white mustard, indian mustard, rocket and all vetch species, 80 plants/m^2 for rape, radish, turnip rape, turnip and pea, 70 plants/m^2 for soya bean and lupin, 40 plants/m^2 for faba bean. In mixtures, sowing densities were half of the corresponding sole crop density of each species (50% crucifer: 50% legume in each mixtures) to create a substitutive design. Seeds of both species were mixed together before sowing to ensure that they were mixed in the row. An experimental seeding machine, similar to a drill-seeder, was used for sowing and the seeds were also mixed with sterile ryegrass to obtain a homogenous mixed-plant cover crop. Sowing was carried out with a row width of 15 cm and a sowing-depth ranging from 1.5 to 2 cm. The proportion of each sown species was controlled during the cover crop emergence phase. Irrigation was applied after sowing to L2014, L2015 and V2014 to ensure homogeneous emergence and establishment of cover crops. No irrigation was applied in V2015 because rainfall was sufficient for plant emergence. Irrigation and fertilization were not applied during the growing period to mimic "normal farming conditions". Shallow tillage was performed at both sites before sowing the cover crop. Weed control was performed in L2014 and L2015 with an herbicide sprayed in September due to strong weed emergence, but no herbicides were applied in V2014 and V2015, which corresponded to normal farming conditions. On bare soils, only manual weeding was performed, to measure the potential nitrate catch crop effect of cover crops.

2.2. Plant and soil sampling

Cover crops were harvested ca. 3 months after sowing (Table 7), which is consistent with the usual practice of cover crop in conventional tillage systems where cover termination is done before sowing the subsequent winter crop. All above-ground biomass of legumes, and all above-ground and root biomass of crucifers, was collected from 1 m^2 per replicate. Crucifers roots were collected to a depth of ca. 30 cm. Samples were washed, dried at 80°C for 48 h, weighed and ground to measure total C and N concentrations via elemental analyses based on the Dumas method (Elementar MicroVario Cube, Germany). Wet clay and loam soils at the date of sampling did not permit us to properly sample legumes roots in our experiments due to a great proportion of fine roots. Then, to estimate N content in legume roots, data for

nitrogen shoot: root ratio measured in multiple legume species were obtained from an experiment conducted at the INRA Toulouse site in 2012 (see Tribouillois et al., 2015 for experimental field details). Based on these data, we calculated two coefficients called k in order to estimate the N acquired and the C:N ratio of the whole legume plant while we only had shoots data:

k_N_R = [N acquired by the whole plant] / [N acquired by the shoots].

k_CN_R = [C: N ratio of the whole plant] / [C:N ratio in the shoots].

Value of the coefficients used are indicated in Table 10.

Soil profiles for SMN analysis were sampled at sowing to depths of 60 cm (V; shallow soil) and 90 cm (L; medium-deep soil). SMN varied from ca. 40 kg N ha^{-1} in L2015 to 139 kg N ha^{-1} in V2014 (Table 7). To characterise the nitrate catch crop service, soil samples were also taken from each plot one day after sampling cover crop shoot and root biomass. These soil samples were taken only in Lamothe site on both years due to limiting labour force in La Vannelière site. Soil samples were taken with a hydraulic core drill in two or three cores of 30 cm (two replicates per plot) and then mixed to represent the soil profile. Nitrate and ammonium were extracted with 1M KCl and were measured with a continuous flow auto-analyser (Skylar 51,000, Skalar Analytic, Erkelenz, Germany).

2.3. Indicators used to characterise mixture performances

We used the Land Equivalent Ratio (LER) indicator to assess the complementarity and competition of crucifer and legume species for resource acquisition and their performance relative to sole crops. LER, defined as the area of a sole crop required to reach the same biomass as a multi-species mixture (Willey, 1979), is the sum of the partial LER (LERp) of each mixed species. We used the LER to assess N acquisition in the same way as Bedoussac and Justes (2011) did for wheat-pea intercrops:

$$LER = LERpC + LERpL \quad \text{(Eq. 1)}$$

$$LERp = N_{acqMIX} / N_{acqSC} \quad \text{(Eq. 2)}$$

where LERpC and LERpL are respectively the partial LER of crucifers and legumes in the mixtures. N_{acqMIX} is the N acquired by each species (crucifer or legume) in the mixture, and N_{acqSC} is the N acquired by the same species as a sole crop.

LER>1 indicates that the multi-species mixture used resources more efficiently than the species grown as sole crop. In contrast, LER<1 indicates that competition was greater than complementarity for resource acquisition, while LER=1 indicates that the balance of plant interaction is null and no advantage was obtain by growing species in mixture. In a substitutive design (50% of each sole crop species), LERp>0.5 indicates that, at the plant level, the species acquired more N in the mixture than as a sole crop, while LERp<0.5 means that the species is negatively affected by interspecific interactions in the mixture.

The amount of N mineralised for the subsequent cash crop within 6 months ($N_{mineralised}$) was estimated following the equation developed by Justes et al. (2009) for cover crop species with a C:N ratio range of 9.5-34:

$$N_{mineralised} = N_{acq} \times N_{\%ava} \quad \text{(Eq. 3)}$$

where $N_{\%ava} = 0.72 - [26.57 \times \text{C: N ratio}] / 1000$, which corresponds to the percentage of N acquired by cover crops (shoots + roots) that will be mineralised and available for the subsequent cash crop. $N_{mineralised}$ corresponds to the green manure service provided by the cover crop after its incorporation into the soil (Tribouillois et al., 2016).

2.4. Statistical analysis

Statistical analysis was performed on four measured variables: 1) N acquired (shoots + roots), 2) C:N ratio, 3) Mineralised and 4) SMN. Analysis of variance (ANOVA) was used to evaluate effects of site, year and cover crop type (sole crop or mixture) on each variable. Tukey post-hoc test was used to distinguish differences among cover crop types for each site-year. Normality was tested with the Shapiro-Wilk test. Nonparametric Wilcoxon tests were performed: 1) to test the differences between cultivars of individual species for each of the four variables analysed; 2) and to test the significance of differences in LER between covers and its eventual difference from the value of 1.0, as well as those between LERpC and LERpL and 0.5. For all data analysis, significant differences among treatments were identified at the 0.05 probability level of significance. Statistical analyses were performed with R software (R Core Team, 2016).

Table 9. ANOVA probabilities for nitrogen (N) acquisition, soil mineral nitrogen (SMN) and estimated mineralised N (green manure effect) as influenced by two experimental sites (Lamothe or la Vannelière), the two years (2014 or 2015) and the cover crop type (crucifer sole crops or legume sole crops or mixtures) as fixed effects. Abbreviations ns, *, **, *** indicate non-significant, or significant differences at p=0.05, 0.01, or 0.001, respectively. SMN was assessed at only one site (Lamothe). NA indicate non applicable (no data available to test this interaction).

Fixed effect	**N acquired**	**SMN**	**$N_{mineralised}$**
Site	**	NA	**
Year	***	***	*
Cover crop type	***	***	***
Site × year	***	NA	*
Site × cover crop type	***	NA	**
Year × cover crop type	ns	ns	ns
Site × year × cover crop type	ns	NA	ns

Table 10. C and N indicators of cover crops for shoot and root allocation on the termination date of crucifer and legume species. Values correspond to the mean of the four experimental sites-years for crucifers and from the experiment of Tribouillois et al (2015) for legumes. N_R:N_T is the proportion of total N acquired by the whole plant (shoots + roots) that is found in the roots. C_T:N_T is the C:N ratio of the whole plant. Coefficient k is used to estimate the N acquired by (or C:N ratio) of the whole plant when only the N acquired (or C:N ratio) in the shoots is known. k_N_R = N acquired by the whole plant / N in the shoots. k_CN_R = C:N ratio of the whole plant / C:N ratio of the shoots. Numbers in brackets represent standard errors.

Species	**N_R:N_T**	**C_T:N_T**	**k_N_R**	**k_CN_R**
rape	0.09 (0.01)	22.0 (1.0)	1.10 (0.01)	1.20 (0.01)
white mustard	0.08 (0.01)	24.2 (1.4)	1.09 (0.01)	1.22 (0.03)
Indian mustard	0.19 (0.02)	19.5 (1.4)	1.23 (0.02)	1.30 (0.06)
Ethiopian mustard	0.09 (0.00)	25.8 (1.5)	1.10 (0.01)	1.33 (0.03)
turnip	0.38 (0.01)	23.7 (0.6)	1.62 (0.03)	1.28 (0.09)
turnip rape	0.26 (0.03)	19.8 (0.7)	1.37 (0.05)	1.23 (0.05)
radish	0.28 (0.01)	22.4 (1.1)	1.39 (0.02)	1.25 (0.03)
rocket	0.07 (0.00)	14.8 (1.1)	1.08 (0.01)	1.02 (0.01)
Mean crucifers	***0.18 (0.04)***	***21.5 (1.15)***	***1.25 (0.06)***	***1.23 (0.03)***
Egyptian clover	0.05 (0.02)	15.4 (2.4)	1.06 (0.02)	1.05 (0.03)
crimson clover	0.05 (0.02)	12.2 (0.8)	1.05 (0.02)	1.01 (0.01)
common vetch	0.05 (0.03)	10.2 (1.4)	1.06 (0.03)	1.00 (0.01)
purple vetch	0.02 (0.01)	11.0 (1.4)	1.02 (0.01)	1.01 (0.00)
hairy vetch	0.02 (0.01)	10.2 (1.6)	1.02 (0.01)	1.01 (0.00)
pea	0.03 (0.01)	9.3 (0.1)	1.03 (0.02)	1.01 (0.00)
soya bean[a]	0.07 (0.05)	22.5[a] (4.2)	1.09 (0.00)	1.01 (0.00)
faba bean	0.14 (0.07)	12.0 (2.5)	1.18 (0.10)	1.02 (0.01)
lupin	0.12 (0.08)	10.2 (2.2)	1.16 (0.10)	0.98 (0.02)
Mean legumes	***0.06 (0.03)***	***12.6 (1.3)***	***1.07 (0.04)***	***1.01 (0.05)***

[a] Because soya bean were not inoculated with *Rhizobium japonicum*, they did not fix N_2.

3. Results

3.1. Main results of analysis of variance for the effects tested

ANOVA results suggest that site, year and cover crop type (sole crop or mixture) significantly influenced i) cover crop N acquisition, ii) SMN and iii) estimated N mineralisation (Table 9). The interactions of site × year and site × cover crop type were found significant while those of year × cover crop type and site × year × cover crop type were not significant. Furthermore, nonparametric Wilcoxon test carried out for comparing cultivars of a same species on a given site, indicated that very few statistical differences of performances occurred both in sole crops and mixtures. The only cases where differences were shown were in L2014 for i) Ethiopian mustards, where cv *Carbon* had a lower SMN (P=0.01) than cv *Utopia* in mixtures and a lower N acquired in sole crop (P=0.05) and ii) hairy vetch cv *Massa* had a higher $N_{mineralised}$ than cv *Savane* only in mixtures (P=0.05). As a result of this rare occurrence of varietal differences, we further analyzed the results by considering the cultivars as replicates of the species in order to have more robust and generic conclusions on mixture versus sole crop performances. This approach does not suggest that cultivar variability is not of interest, but in our conditions this was not the main factor driving the most remarkable results.

3.2. Importance of including crucifer roots when estimating biomass and N uptake

Among the four sites, the proportion of acquired N contained in roots ranged from 0.07 (rocket) to 0.38 (turnip) for crucifers and 0.02 (purple vetch) to 0.14 (faba bean) for legumes (Table 10). The coefficient k_N_R, used to estimate N acquired by the whole plant only knowing the N content in shoots, ranged from 1.08-1.61 for crucifers and from 1.02-1.18 for legumes. Crucifers with large tuberous roots, such as turnip rape, radish, and turnip, contained much of their biomass in their roots and, among the species tested, had the most acquired N in their roots, highlighting the importance of sampling roots to accurately estimate the amount of N these species acquired. The coefficient k_CN_R, used to estimate the C:N ratio of the whole plant knowing the C:N ratio of the shoots, ranged from 1.06-1.32 for crucifers and 0.98-1.05 for legumes (varying little from 1.0 for the latter). The values of the two coefficients demonstrated the importance of including roots when estimating total N acquired and the C:N ratio of the whole plant in order to estimate more accurately the N mineralised from cover crop residues and available for the subsequent crop. Results that follow are presented for the whole plant (shoots + roots).

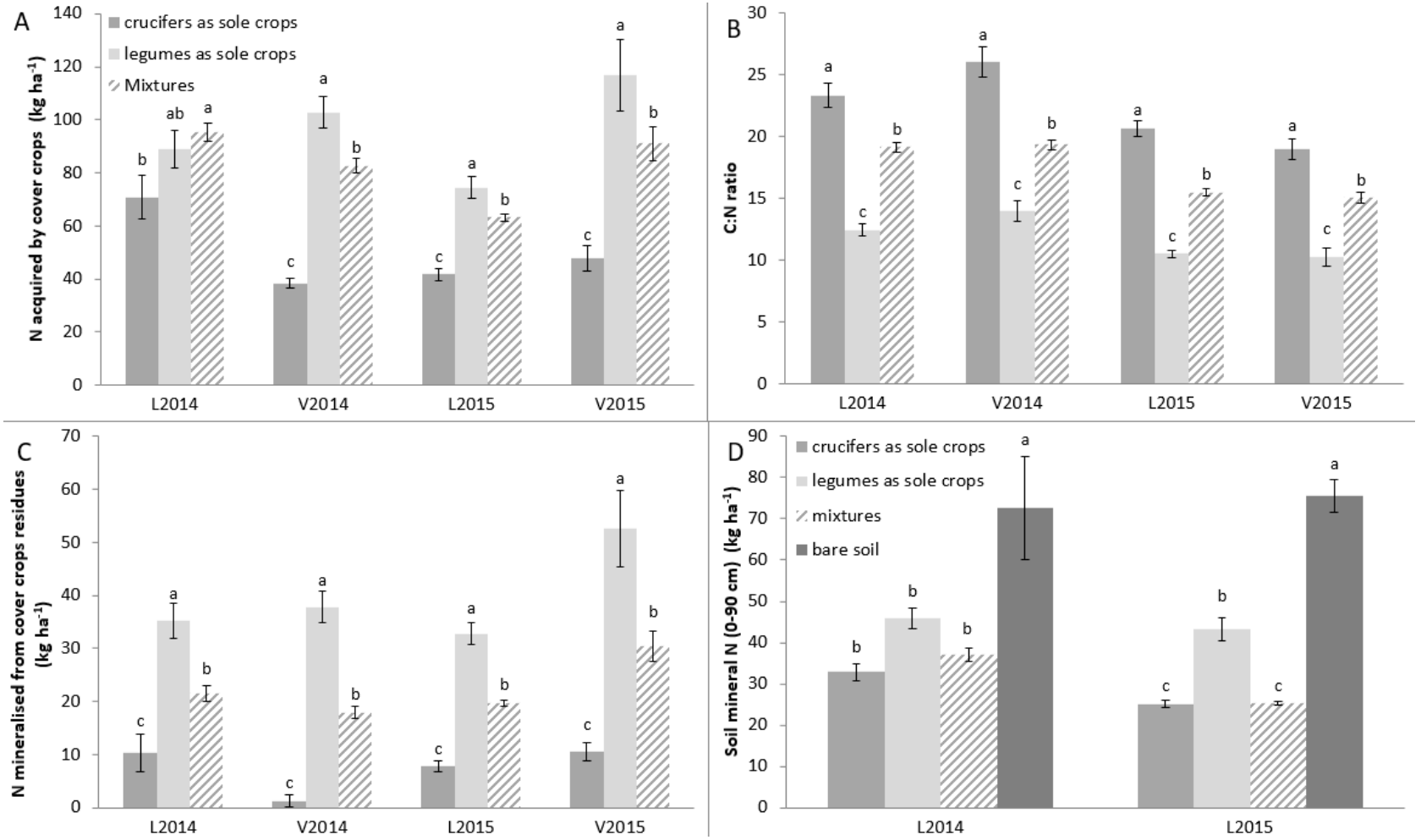

Figure 10. Cover crop performances measured for crucifers and legumes in sole crops and mixtures on the termination date: A) N acquisition of the whole plant; B) C:N ratio of the whole plant; C) calculated N mineralisation from cover crop residues after termination, corresponding to the N green manure service; D) Mean soil mineral N content in soil to a depth of 90 cm, corresponding to the nitrate catch crop service, compared to that of bare soil. Each value represents the mean of all treatments for a given site. Different letters (a, b, c) indicate treatments with significant differences at $P<0.05$. Bars represent standard errors.

3.3. N acquisition of cover crops

By botanical family (crucifer vs. legume), the mean amount of N acquired by crucifer sole crops was 50 kg N ha^{-1} and varied among sites from 38 kg N ha^{-1} in V2014 to 71 kg N ha^{-1} in L2014, while the mean value of N acquired by legume sole crops reached 97 kg N ha^{-1} and varied from 81 kg N ha^{-1} in L2015 to 115 N ha^{-1} in V2015. The mean amount of N acquired by mixtures was 83 kg N ha^{-1} and ranged from 64 kg N ha^{-1} (in L2015) to 95 kg N ha^{-1} (in L2014). At both sites, mean amounts of N acquired by mixtures were significantly higher than those of crucifer sole crops and, except in L2014, significantly lower than those of legume sole crops (Figure 10A).

Concerning species performances, as a sole crop, radish was the crucifer that acquired the most N, taking up 38 kg N ha^{-1} in L2015 to 100 kg N ha^{-1} in L2014 (Figure 11A). Mixtures with white mustard acquired the most N, ranging from 61 kg N ha^{-1} in L2015 to 115 kg N ha^{-1} in L2014 (Figure 11A). At both sites, mixtures acquired more N than each corresponding crucifer sole crop, except mixtures with radish in L2014 due to the small amount of N acquired by hairy vetch (cv. Savane) when intercropped with crucifers (Figure 11A). Among legume sole crops, common vetch acquired the most N, from 76 kg N ha^{-1} in L2015 to 161 kg N ha^{-1} in V2015 (Figure 11B). Mixtures with common vetch also acquired large amounts of N, from 60 kg N ha^{-1} in L2015 to 115 kg N ha^{-1} in V2015. Among years and sites, no significant difference in N acquired was observed between legume species as sole crops or in mixtures tested here, as only 7 mixtures out of 24 acquired more N than their corresponding legume sole crop (Figure 11B).

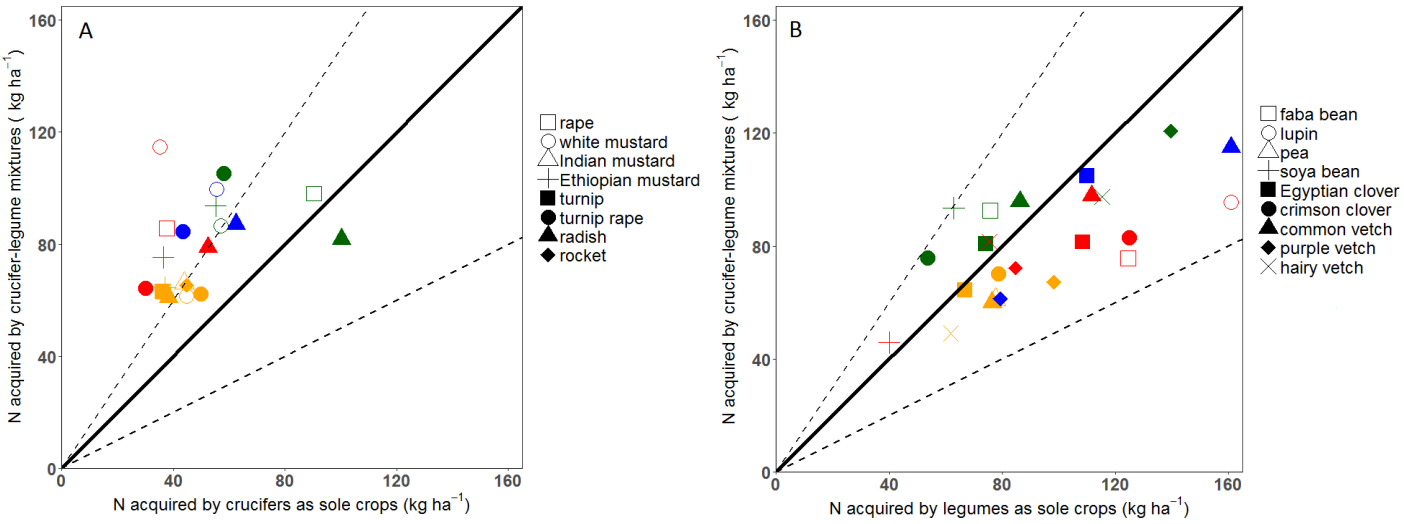

Figure 11. N acquired (kg ha^{-1}) by mixtures (crucifer and legume) as a function of mean N acquired by the corresponding (A) crucifer sole crop or (B) legume sole crop. Each point represents the mean of species cropped as a SC (x-axis) or the mean of mixtures including the species (y-axis). The solid line represents y=x. Dotted lines represent y=1.5y and y=0.5x. Each point represents the mean of mixtures. Colours vary by experimental site (V2014 in red, V2015 in blue, L2014 in green, L2015 in yellow).

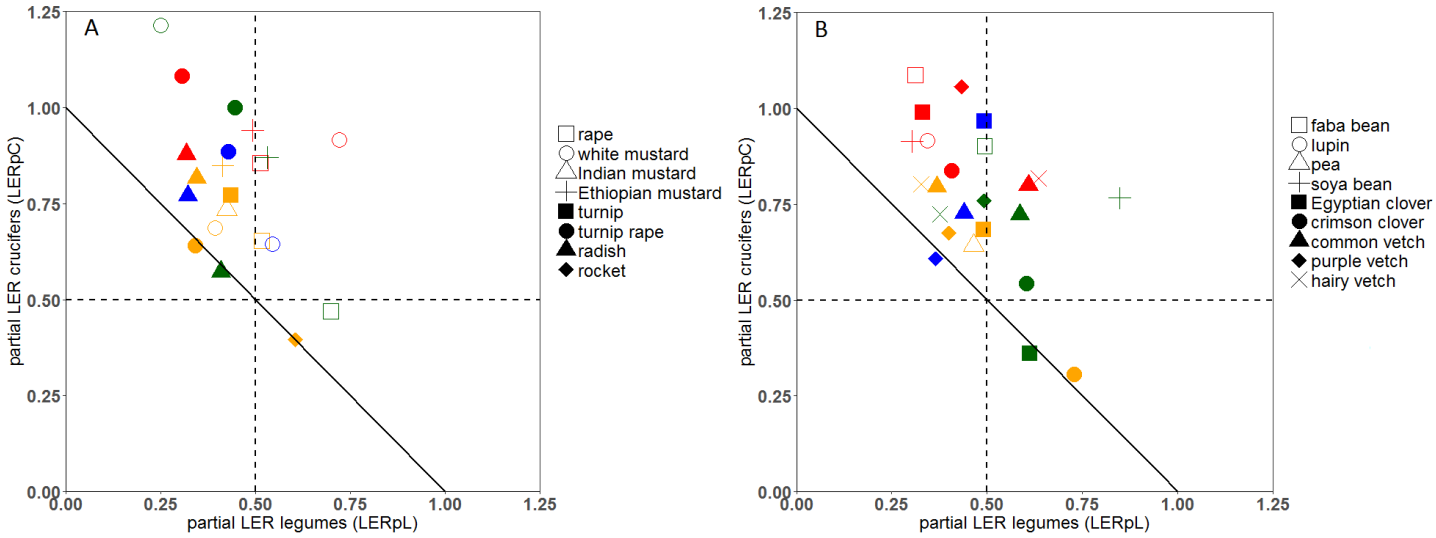

Figure 12. Partial Land Equivalent Ratios (LER) for N acquisition of crucifers (LERpC) as a function of that for legumes (LERpL) according to (A) crucifer species or (B) legume species (B). Each point represents the mean of species cropped in mixtures. The solid line represents y=x. Dotted lines represent LERpC=0.5 and LERpL=0.5. Colours vary by experimental site (V2014 in red, V2015 in blue, L2014 in green, L2015 in yellow).

3.4. Plant interactions in mixtures: complementarity and competition between species

Mean LER was 1.22 for all mixtures and was significantly greater than 1 in all experiments, ranging from 1.15 in L2015 to 1.37 in V2014 (Table 11), indicating that mixtures used available N resources more efficiently than either corresponding sole crops. Mean LERpC was 0.75 for all mixtures and was significantly greater than 0.5 at both sites, ranging from 0.70 (in L2014) to 0.89 (in V2014). This indicates that crucifers tended to benefit from multi-species mixtures, growing more than they did as sole crops. Mean LERpL was 0.5 for all mixtures for all experiments, ranging from 0.42 (in L2015) to 0.56 (in L2014) (Table 11); thus, legumes did not benefit from mixtures in their use of abiotic resources. Then LER higher than 1 was due to crucifer performances in mixtures with legumes.

Among crucifer species (Figure 12A), LER was significantly greater than 1 for mixtures with white mustard (1.19), Ethiopian mustard (1.35), Indian mustard (1.16), turnip rape (1.19) and rape (1.27). LER did not significantly differ from 1.0 for mixtures with radish (1.1), turnip (1.19), and rocket (1.00) (Figure 12A). Most crucifer species had a LERpC significantly greater than 0.5, except for rocket (0.40) (Figure 12A).

Crucifer species competed differently with the associated legume in mixtures (Figure 12B). LERpL of legumes was significantly less than 0.5 when intercropped with radish (0.35) and turnip rape (0.39) but not significantly different from 0.5 when intercropped with rape (0.59), rocket (0.61), turnip (0.44), white mustard (0.44) or Ethiopian mustard (0.48) (Figure 12A). The strongest competition was measured in L2014, when radish was intercropped with hairy vetch (cv. *Savane*) whose LERpL was very low (0.19); however, this trend was not observed in the three other experiments.

Table 11. Mean partial Land Equivalent Ratio (LER) of crucifers (LERpC), legumes (LERpL) and LER for N acquisition measured for all cover crops. Each value represents the mean of species mixtures for experiments conducted in 2014 and 2015 are referred to as L2014 and L2015, respectively, at Lamothe (L) and as V2014 and V2015, respectively, at La Vannelière (V). Letters (a, b) indicate homogeneous groups tested within columns for the four site × year treatments at $P<0.05$. Asterisks indicate that LERp and LER are significantly greater than 0.5 and 1.0, respectively. Numbers in brackets represent standard errors.

Site	LERpC	LERpL	LER
L2014	0.70 (0.06) a*	0.56 (0.04) a	1.24 (0.04) a*
V2014	0.89 (0.02) b*	0.48 (0.02) a	1.37 (0.02) a*
L2015	0.71 (0.05) a*	0.42 (0.02) a	1.13 (0.02) a*
V2015	0.77 (0.02) ab*	0.43 (0.02) a	1.21 (0.06) a*
Mean	**0.75 (0.02) ***	**0.47 (0.01)**	**1.22 (0.02) ***

3.5. N mineralised from cover crop residues: green manure service

The percentage of N acquired by cover crops that would be mineralised for the subsequent cash crop (i.e. the green manure service) was estimated from the amount of N acquired in roots and shoots and the C:N ratio. The mean C:N ratio of crucifer sole crops was 21.5 and was greater than 15 for all crucifer species except rocket. This threshold of 15 is considered the threshold between net N mineralisation (C:N ratio <15) and net N immobilisation (C:N ratio >15) for 6 months after incorporating cover crop residue into the soil. The mean C:N ratio of legume SC was 12.6 and was less than or equal to 15 for all legume species except soya bean, which did not nodulate under study conditions (Table 10). The mean C:N ratio of mixtures was 17.4 at both sites and was significantly higher than that of legume sole crops but significantly lower than that of crucifer sole crops (Figure 10B).

By botanical family, the mean N green manure service provided by crucifer sole crops residues was only 7.5 kg N ha^{-1}, ranging from nearly zero (in V2014) to 10 kg N ha^{-1} (in V2015) among experiments, while that provided by legume sole crops residues was 40 kg N ha^{-1}, ranging from 35 kg N ha^{-1} (L2015) to 54 kg N ha^{-1} (V2015). That provided by mixtures residues was 22 kg N ha^{-1}, ranging from 18 kg N ha^{-1} (V2014) to 30 kg N ha^{-1} (V2015). For each experiment, the N green manure service provided by mixtures was significantly higher than those of crucifer sole crops but significantly lower than those of legume sole crops (Figure 10C).

Among crucifer species as sole crops, the estimated N green manure service ranged from -6 kg N ha^{-1} (i.e. net N immobilisation) for white mustard in V2014 to 20 kg N ha^{-1} (i.e. net N mineralisation) for radish in L2014. In contrast, mixtures always provided net N mineralisation, ranging from 9 kg N ha^{-1} (L2014) to 35 kg N ha^{-1} (V2015) for mixtures with white mustard. At both sites, each mixture released more N than the corresponding crucifer SC, except for radish in L2014 (Figure 13). Overall, no difference in estimated N green manure service was observed between mixtures and crucifer sole crops. The green manure service exceeded 10 kg N ha^{-1} for 7 of 21 crucifers as sole crops and 20 of 21 mixtures.

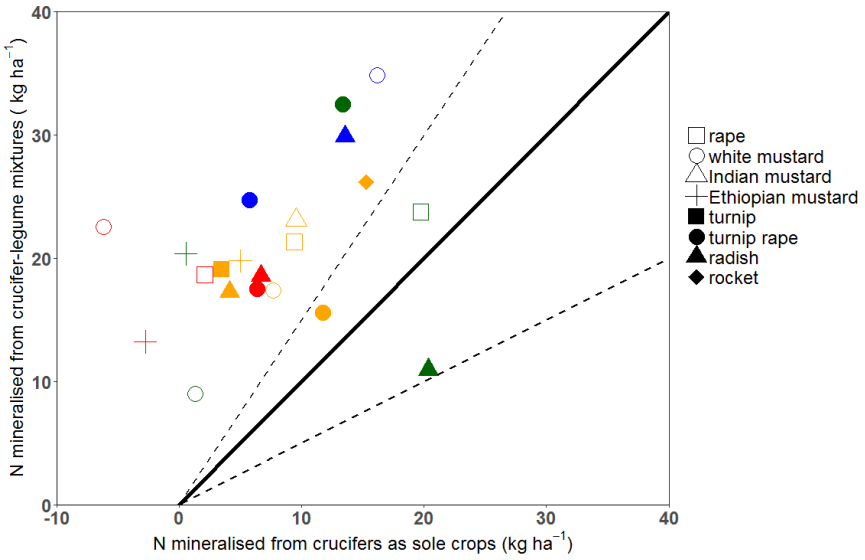

Figure 13. Total estimated N mineralised (kg N ha-1) within 6 months from cover crop residues of crucifer- legume mixtures as a function of N mineralised by crucifer sole crops. The solid line represents y=x. Dotted lines represent y=1.5y and y=0.5x. Each point represents the mean of species cropped as a sole crop (x-axis) or the mean of mixtures with the corresponding species (y-axis). Colours vary by experimental site (V2014 in red, V2015 in blue, L2014 in green, L2015 in yellow).

3.6. SMN at termination of cover crop in autumn: catch crop service

SMN in the upper 90 cm of the soil profile was measured on the date of cover crop termination in L2014 and L2015 (Figure 14). As rainfall was low during the growing period in both years (140 mm in L2014 and 90 mm in L2015), simple water balance calculations indicated that no drainage occurred because actual evapotranspiration was higher than rainfall from sowing to termination date. Consequently, by measuring the difference in SMN between bare soil (without crop N uptake) and cover crops in early November, we estimated the potential catch crop service based on the decrease in SMN due to N uptake before winter, when nitrate leaching normally occurs.

The SMN of bare soils in early November was 73 and 76 kg N ha^{-1} in L2014 and L2015, respectively. In comparison, mean SMN was significantly lower under crucifer SC (reduced SMN by 51% in L2014 and 70% in L2015), legume SC (reduced SMN by 37% in L2014 and 43% in L2015) and, importantly, mixtures (reduced SMN by 48% in L2014 and 70% in L2015). Thus, mixtures of crucifer-legume tested here were globally as effective as crucifer sole crops in decreasing SMN in both years (Figure 10D).

At the species level, relative decreases in SMN compared to values under bare soil for crucifer sole crops ranged from 45% for rape to 59% for Ethiopian mustard in L2014 (Figure

14A) and 58% for rape to 79% for white mustard in L2015, but no significant difference was observed among crucifer species (Figure 5B). In mixtures, the relative decrease in SMN varied from 30% for mixtures with turnip rape to 56% for mixtures with white mustard in L2014 (Figure 14A) and from 59% for mixtures with rape to 70% for mixtures with Ethiopian mustard in L2015 (Figure 14B). For all species, no significant difference was found in SMN between the species in mixtures and as a sole crop (Figure 14). The relative decrease in SMN under legume sole crops ranged from 25% for crimson clover to 56% for pea in L2014 (Figure 14A) and from 30% for crimson clover to 54% for pea in L2015 (Figure 14B). In mixtures, the relative decrease in SMN ranged from 64% for mixtures with crimson clover to 71% for mixtures with hairy vetch in L2014 (Figure 14A) and from 66% for mixtures with crimson clover to 74% for mixtures with hairy vetch in L2015 (Figure 14B).

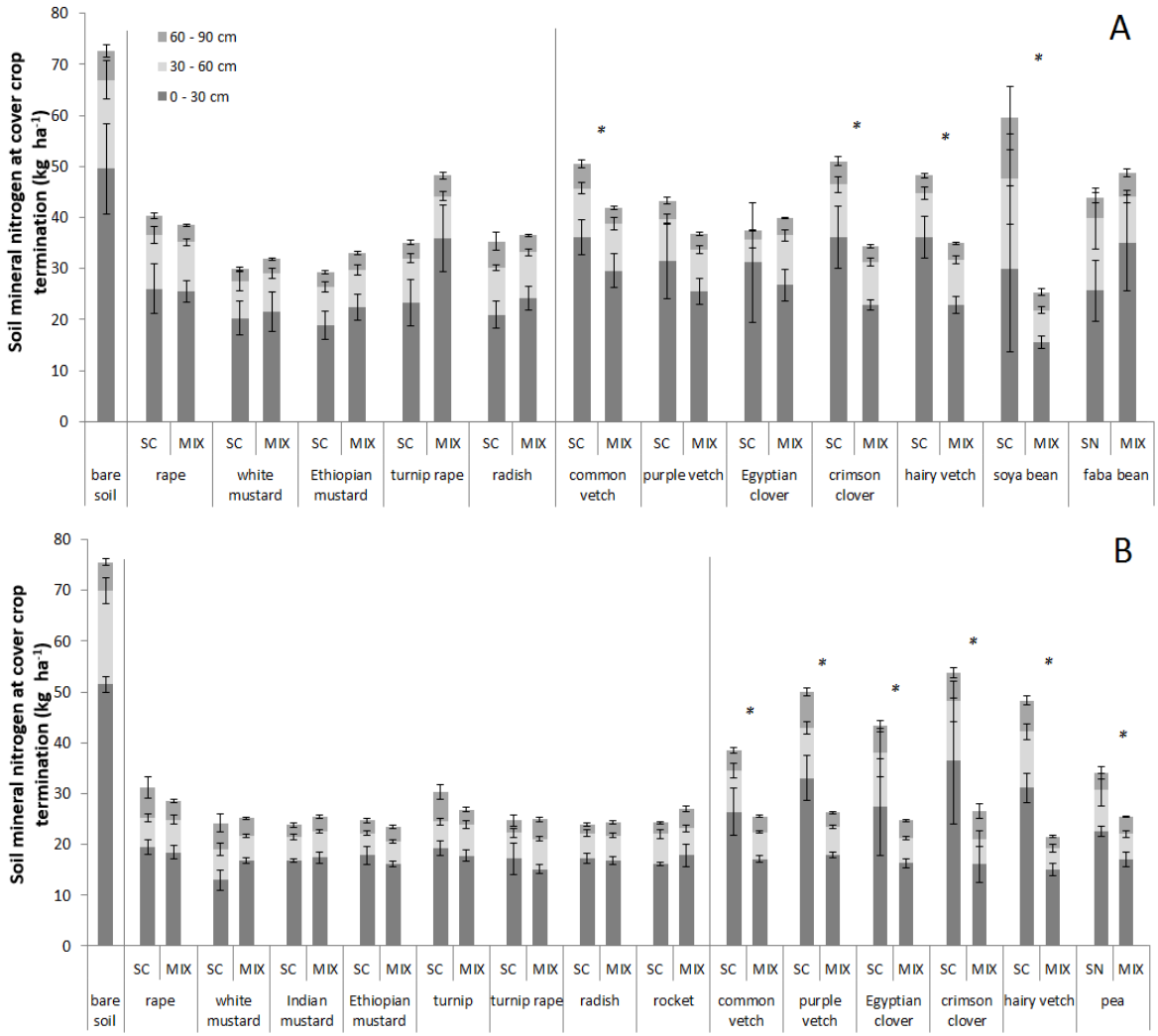

Figure 14. Soil mineral nitrogen (SMN, kg ha-1) from 0-90 cm on the date of cover crop termination at Lamothe in (A) 2014 and (B) 2015. Values correspond to the mean of species sole cropped (SC) or the mean of crucifer-legume mixtures (MIX) with the corresponding species. Bars indicate the standard error. Asterisks indicate a significant difference in SMN between a given species as sole crops and in mixture ($P<0.05$).

4. Discussion

4.1. Crucifer-legume mixtures effectively provide the most nitrate catch crop service

Crucifer-legume mixtures tested here had the same potential nitrate catch crop service as crucifer sole crops in both years and for each mixtures tested. Even though SMN was not measured at the La Vannelière site, we assume that mixtures there would provide nitrate catch crop services based on their effectiveness in acquiring N, as indicated by their whole-plant N contents. Our results are consistent with those of Tribouillois et al. (2016) for turnip rape. We demonstrate the effectiveness of crucifer-legume mixtures for a wide range of species, and combinations of them, which increases the generality of the findings.

This highlights that mixtures produced positive complementarity between crucifer and legume species for decreasing SMN in the soil during autumn, for a wide range of plant architectures and precocities. This agrees with studies that shown mixtures induce deeper and faster root growth in both intercropped species (Li et al., 2006; Tosti and Thorup-Kristensen, 2010), which leads to niche complementarity for N and most likely for water and other nutrients. Consequently, these bispecific mixtures take up the same amount of N than crucifer sole crops, and more than legume sole crops. Our study also confirms the significant catch crop service of a wide range of legume sole crops even though it was lower than that of crucifer sole crops, as ever demonstrated in the literature (Tonitto et al., 2006; Tribouillois et al., 2016a). Thus, it is always more effective to grow a legume cover crop than to rely on bare soil in order to jointly i) recycle N, ii) improve soil chemical fertility and iii) add carbon to the soil via production of cover crop dry matter.

On a species basis, under our study conditions, all crucifer species tested taken up similar amounts of SMN both as sole crops and in mixtures. This indicates that each crucifer species tested could provide a high nitrate catch crop service. Thus, crucifers could be chosen for mixtures not only for their catch crop service but also to provide N green manure service, carbon soil storage, biofumigation properties (Matthiessen and Kirkegaard, 2006) or soil structure, and then provide efficiently multi-ecosystem services. Crucifer species used as cover crops can also be changed each year in a given rotation to avoid pathogen specialisation, which facilitates the design of agroecological cropping systems that are effective from multiple viewpoints and provide multiple ecosystem services.

4.2. Crucifer-legume mixtures as a way to improve the N green manure service provided by cover crops

Our results highlighted a higher green manure service of crucifer-legume mixtures than that of crucifer sole crops and confirm other studies dealing with other non-legume species (Tosti et al., 2014; Tribouillois et al., 2016a). Even though legumes did not benefit in mixtures to better use resources due to crucifer competition, they grew enough within the plant cover to significantly increase the whole cover crop N acquisition and then decrease the C:N ratio of mixtures compared to crucifer sole crops. This generating a more effective N green manure service due to faster and greater N mineralisation of residues, as demonstrated by Justes et al. (2009) for white mustard and rape volunteers. For each site × year and each species, mixtures provided a positive N green manure service, which avoided the net N immobilisation sometimes obtained with crucifer cover crops. This highlights the great benefit of growing together crucifers and legumes in mixtures to increase the N green manure service of cover crops. However, as expected, the green manure service of mixtures was lower than those of legume sole crops due to higher C:N ratios and lower N acquisition, which decreased mineralisation of cover crop residues.

On a species basis, the N green manure service of species and cultivar mixtures varied greatly and was not fully consistent among sites or years, indicating that broader studies under additional soil and climate conditions are required to explore the influence of the latter on cover crop interactions and performances. From a practical viewpoint, regardless of the species and cultivars used, the most effective N green manure service provided by crucifer-legume mixtures will most likely avoid the pre-emptive competition induced by crucifer sole crops compared to bare soil (Thorup-Kristensen et al., 2003). That is an important point in order to avoid the use of more N fertilisers to compensate this effect.

Our study also demonstrated the importance of including the N contained in crucifer roots to estimate the actual N green manure service more accurately. Our study provides new references for estimating the N content of crucifer roots, which enables more accurate assessment of the N green manure effect. For legume cover crops, studies have estimated that roots contain approximately 30% of the total N acquired by kidney hairy vetch, white clover and pea on sandy and silt loam soils (Askegaard and Eriksen, 2007; Kumar and Goh, 2002). Our study found less N in legume roots, indicating that the shoot:root ratio of legume cover crops could also depend greatly on soil type, nutrient availability and climate. Estimates of legume shoot:root N ratio from a wide range of environmental conditions are necessary to

obtain more data about the former and to increase the relevance of assessing the N green manure service of cover crops, without always needing to sample legume roots in the soil.

4.3. Crucifer-legume competition and complementarity

Our study obtained LER values significantly greater than 1 (often reaching 1.3), indicating that crucifer-legume mixtures had effective N acquisition. Crucifers in all experiments benefited from the mixtures, having an LERpC significantly higher than the theoretical value of 0.5. Intra-specific competition for crucifers was higher than inter-specific competition with associated legumes, which enables significant expression of niche complementarity for resource use and/or facilitation processes. The highest LERpC occurred in V2014, which was the wettest site and where SMN was highest at sowing; this result agrees with (Bedoussac and Justes, 2010), who found in bi-species mixtures of durum wheat-winter pea that the pLER of wheat was highest when more N was available. Overall, crucifers are the most effective catch crops because they grow the fastest with a deep root system (Thorup-Kristensen et al., 2003) and are thus highly competitive in acquiring abiotic resources, at least as much as cereals. Our results confirm findings that show LER>1 and LERpC>0.5 for rape-spring pea (Andersen et al., 2005; Szumigalski and Van Acker, 2008) and Ethiopian mustard-chickpea (Lal et al., 2016) mixtures grown for grain yield. Our results also agree with those of mixture studies that include turnip rape (Tribouillois et al., 2016a), white mustard (Wortman et al., 2012) or indian mustard (Wendling et al., 2017). In our study, the three mustard species showed complementarity in optimising N acquisition, unlike results of Wortman et al., (2012), which showed that mustard competed greatly with hairy vetch. We observed that competition with legumes was greater for radish and turnip rape, which have larger roots (taproot) than other species. The radish is one of the fastest growing crucifers, rapidly taking up SMN (Thorup-Kristensen, 2003; Kristensen and Thorup-Kristensen, 2004). Except for the mixtures with radish and hairy vetch (cv. *Savane*) in L2014, no incompatibility was observed in the mixtures tested, despite the high and variable competition for abiotic resources generated by crucifers in the cover crops and the possible allelopathic effect often described in some studies (Gimsing and Kirkegaard, 2009). Even though crucifers clearly grew faster than legumes, these crucifer-legume mixtures were sufficiently complementary to provide the expected multi-ecosystem services.

4.4. Designing effective mixtures to optimise the complementarity of nitrogen ecosystem services

The ability of mixtures to provide both nitrate catch crop and green manure ecosystem services resulted from 1) the combination of complementary species traits (growth rate and N acquisition) and 2) more efficient resource use due to niche complementarity and/or facilitation processes. Neither N recycling service is independent (Tribouillois et al., 2016a), and the degree of achieving one service can affect that of the other, meaning that the design of multi-species mixtures must be consistent with the services desired to create a multi-service cover crop.

Crucifer competition with legumes must decrease to provide a high N green manure service. Non-optimal crucifer density could lead to high competition with legumes and a non-optimal N green manure service. Several studies that investigated grain production indicated that the best complementarity for mustard-legume mixtures occurred when mustard was sown at a density less than 50% of its sole crop density, and when the legume was sown at a density more than 50% of its sole crop density (Kushwaha, 1987). In our experiment, each mixture was sown with a 50%-50% density compared to those of its component in sole crop, but crucifer density must be selected based on catch crop service objectives. We observed that crucifer sole crops provided the same level of nitrate catch crop service as mixtures, which suggests that crucifer density could be decreased in mixtures without a significant loss of the nitrate catch crop service. One way to increase the N green mature service of these mixtures is to increase the proportion of legumes in the cover. Future studies are required to address this idea, e.g. testing a variety of densities of the two species -in order to determine the most effective threshold density-, or mixing more than two species to design the most appropriate species composition with the most suitable complementarity for them to grow together. This would enable an increase in legume density, which could provide a more effective green manure service due to higher N acquisition, by increasing legume N_2 fixation and decreasing the C:N ratio in the mixture.

The N green manure service could have an indirect effect on nitrate leaching depending on the date of cover crop termination. If legumes sole crops are destroyed too early before winter, they can release large amounts of N into the soil in the winter and early spring, when N leaching still occurs. The intermediate level of green manure service provided by mixtures could then be an asset by 1) decreasing the risk of N released too quickly after early termination and, simultaneously, 2) decreasing the risk of net N immobilisation after

incorporation of cover crop residues and avoid the negative effect of pre-emptive competition for the subsequent cash crop. The objective of providing a green manure service via cover crops should thus be analysed by considering that N release occurs dynamically, like nitrate leaching. Then analysing all the processes and dynamics together is necessary to assess the nitrate pollution mitigation service, e.g. by using soil-crop modelling, as demonstrated by Tribouillois et al. (2016).

Beyond the green manure service one can also estimate the amount of N available for the subsequent cash crop. In a dry autumn/winter, crucifer sole crops could increase pre-emptive competition for SMN. This effect must be considered when assessing the influence of cover crops on N availability at the crop-succession time scale. In dry climates, increasing cover crop biomass could decrease available N for the subsequent cash crop due to pre-emptive competition for SMN. A soil-crop simulation model, such as STICS (Brisson et al., 2003), could be used to predict dynamic N budgets under different amounts of cover crop biomass to compare potential N green manure service of cover crops under a wide range of environmental conditions, thereby increasing the generality of experimental studies.

5. Conclusion

Cover crop mixtures composed of one crucifer and one legume species provided as much nitrate catch crop service and more N green manure service as sole crucifer cover crops, which are widely used by European farmers. These levels of nitrate catch crop and green manure services were validated for a wide range of crucifer and legume species that differ in their architecture, precocity and C:N ratio. Both N-related ecosystem services were based on combinations of two species that had suitable complementarity for resource capture and use. Even though crucifers competed strongly with legumes for abiotic resources, no mixtures were found to be incompatible with legume development, and legume growth was always high enough to provide a positive N green manure service, due to consistently positive net N mineralisation from cover crop residues. Crucifer-legume mixtures yielded more effective N-related ecosystem services than crucifer sole crops, and for a wide range of species. As a consequence, other eco-system services are improved in species mixtures such as soil coverage and carbon storage into soil due the greater growth and biomass produced. This supports the idea that crucifer-legume mixtures are providing multi-ecosystem services beyond N management services. Then it could be recommended to diversify the species

included in cover crops in European temperate cropping systems in order to provide high level of multi-ecosystem services.

Chapter 4. Crucifer-legume cover crop mixtures provide effective sulphate catch crop and sulphur green manure services.

This chapter is published as:

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Graphical representation of research objectives:

In this chapter for various cultivars of nine crucifer and height legume species we ask the questions:

- Do mixtures reach the same nitrate catch crop effect and green manure services than pure crucifers despite inclusion of legumes?
- Are we able to highlight combinations of species that are either highly compatible or incompatible to mutualize services linked to S cycle?

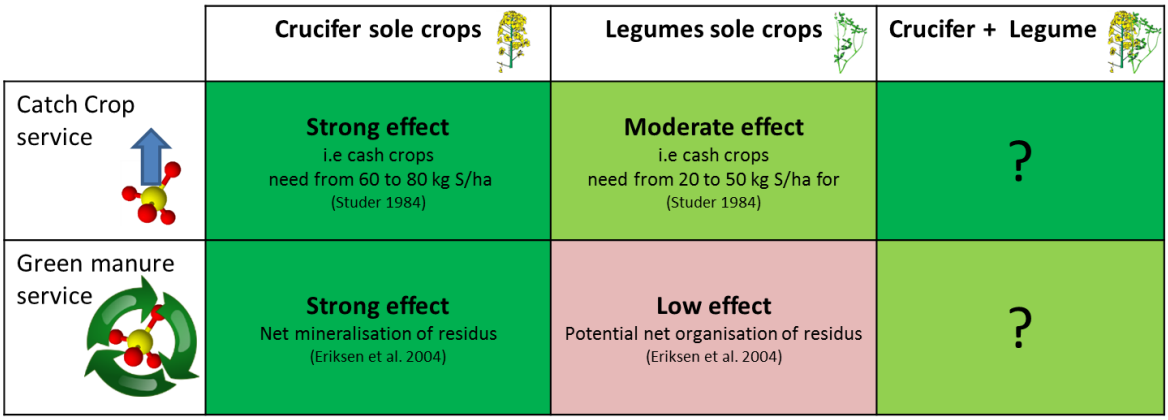

Figure 15. Summarized scheme of questions raised by the chapter 4

1. Introduction

Sulphur (S) availability is a growing concern in many agricultural systems due to their increasing S deficiency in developed countries since the end of the 1980s. This S deficiency is attributed to 1) a drastic reduction in SO_2 emissions from fossil fuel industries (-85%), 2) the use of NPK fertilisers with low S contents, 3) a decrease in the S content of pesticides and fungicides, 4) increased S "exportation" in cash crops due to higher yields, and 5) a decrease in the use of farmyard manure (Scherer, 2001). Several authors indicate that S deficiency could restrict the yield and quality of conventional and organic production (Zhao et al., 2002; Guzys and Aksomaitiene, 2005). Widespread symptoms of macroscopic S deficiency have been identified in oilseed rape since 1986, in cereals since 1992, and in sugar beet since 1995 (Haneklaus et al., 2008).

One straightforward solution to S deficiency is to apply S fertiliser to most crops (Pedersen et al., 1998), but S fertilisation can have adverse economic and environment impacts. For example, sulphate leached in water that drains below the rooting depth is the main S output when soil is left bare during fallow periods (Eriksen, 2008), and S fertilisation could increase this phenomenon if not well managed (dose and timing). When grown during the autumn-winter period between two cash crops in annual rotation, cover crops can be an effective solution to decrease sulphate leaching (S catch-crop ecosystem service) due to their ability to uptake soil mineral sulphate (SMS) (Eriksen and Thorup-Kristensen, 2002), as widely demonstrated for other anions, such as nitrate (Constantin et al., 2011). If incorporated into the soil, cover crops can also provide a S green-manure service once decomposition and mineralisation release the S in their tissues to the subsequent cash crop. N and S mineralisation from cover crop residues was found to be inversely proportional to the carbon (C):S ratio of the cover crops (Eriksen et al., 2004). These S catch-crop and green-manure services are a potential solution to improve S recycling in agroecosystems by addressing S supply issues and replacing or reducing S fertiliser use in low-input conventional farming, which could be a relevant solution for organic farming.

Among cover crops, crucifers uptake the most SMS (and nitrate), due to their rapid increase in root depth and density (Eriksen and Thorup-Kristensen, 2002; Thorup-Kristensen, 2001; Thorup-Kristensen et al., 2003), which allow them to meet their high S requirements for production of S-based amino acids and glucosinolates (GSL) (Falk et al., 2007). Due to a lower C:S ratio than that of cereals (120-600) or legumes (150-350), crucifers (40-150)

provide large amounts of mineralised S to the subsequent main cash crop (Eriksen et al., 2004; Niknahad-Gharmakher et al., 2012). Thus, crucifers may be the best cover crop for providing S catch-crop and green-manure ecosystem services. Compared to legume cover crops, however, crucifers provide a lower N green-manure service that can sometimes lead to net N immobilisation (e.g Thorup-Kristensen et al., 2003; Couedel et al., 2018). "Substitutive" bispecific crucifer-legume mixtures (50%-50%) as cover crops were found to improve N green-manure services compared to crucifer sole cover crops (Tribouillois et al., 2016; Couedel et al., 2018). Legumes provide large amounts of mineralised N to the subsequent cash crop due to a low C:N ratio and the large amounts of N acquired from the soil and the atmosphere (Thorup-Kristensen et al., 2003). Unlike their provision of the N green-manure service, however, legume sole cover crops most likely provide a lower S green-manure service than crucifer sole cover crops because of their lower S uptake and higher C:S ratio, which may lead to net S immobilisation instead of net S release (Eriksen, 2005).

When selecting a cover crop strategy, in particular the choice of species, farmers usually prioritise enhancing N-related ecosystem services, especially in "nitrate sensitive areas," where the European Nitrate Directive (1991) requires the planting of cover crops. Unlike for N capture, few studies focus on understanding how interactions (complementarity and competition) in crucifer-legume mixtures influence S capture. The influence of complementarity on N capture is well documented for crucifer-legume cover crop mixtures (e.g. Wortman et al., 2012; Couedel et al., 2018). Crucifers benefit from the mixture by taking up more soil mineral nitrogen, while legumes experience competition but rely on N_2 fixation to meet N requirements (Tribouillois et al., 2016). The influence of interactions on S capture could differ, however, because legumes also rely on soil mineral S to fulfil their S requirements but require lower amounts of S in comparison to crucifers, which are the cropped species the most demanding in S. Unlike their use of N_2 fixation to meet N requirements, legumes rely only on SMS to meet S requirements. Furthermore, crucifers could be an unsuitable companion in cover crop mixtures because they compete strongly for light, water, and nutrients – including S – and can have an allelopathic effect on legumes by producing GSL that can be exuded and transformed in the soil into biocides, such as isothyocianates (Chew, 1988b; Matthiessen and Kirkegaard, 2006). In a laboratory experiment, Genard (2016) observed strong competition for S uptake between oilseed rape

and legumes. To our knowledge, no field experiment has been published that assessed competition for S between a crucifer and a legume in a cover crop mixture.

Competition and the relative percentage of crucifers and legumes determine the S green-manure service because they influence the total amount of S taken up and the C:S ratio of the species mixture. Competition for abiotic resources differs greatly among crucifer species due to differences in their shoot and root architecture and development, which can lead to dynamic interactions with a legume during growth in a cover crop mixture. Few studies have explored cover crop performances based on genetic variability in crucifers and under a variety of growing conditions (e.g. Wortman et al., 2012; Tribouillois et al., 2016). There is thus a need to study and explain how interactions in a wide variety of crucifer-legume mixtures influence S uptake.

From the practical viewpoint of cover cropping, despite the great interest in crucifer cover crops to enhance S ecosystem services, a lack of knowledge exists about shoot and root S contents of plants (Wendling et al., 2016). Few experiments have considered root biomass and root S content, even though the latter can represent a high proportion of the total S taken up by certain crucifers, such as radish and turnip due to their taproots. As roots can have a different C:S ratio than shoots, they must be included to accurately predict the S green-manure service of cover crop residues for the subsequent main cash crop.

The aim of our study was to analyse the S ecosystem services which bispecific crucifer-legume mixtures provide by testing a wide variety of species – including several crucifer cultivars (cv.) – to obtain levels of inter-specific interactions. Two key aspects of S-related cover-crop services were assessed: 1) levels of S green-manure and catch-crop ecosystem services, for both sole cover crops and cover crop mixtures, and 2) the type of interaction between the two species (complementarity or competition) involved in S uptake. Three hypotheses for using cover crop mixtures in short fallow periods (summer to mid-autumn growing period) were tested:

1) Bispecific crucifer-legume mixtures have the same S uptake as crucifer sole cover crops, thus providing the same S catch-crop service, mainly due to niche complementarity of root systems when exploring the soil.

2) Bispecific crucifer-legume mixtures provide more than 50% of the S green-manure service provided by crucifer sole cover crops due to a high proportion of crucifer biomass.

3) The most suitable compromise between S catch-crop and S green-manure services depends on inter-specific interactions that maximise the complementarity of both species, which can be achieved by specific species mixtures which provide the targeted services most effectively.

Recent studies have highlighted that many factors, particularly climate, soil type and N availability at sowing, have the greatest influence on the performances of species mixtures (e.g. Tribouillois et al., 2016). Accordingly, we assessed performances of crucifer-legume bispecific mixtures by conducting field experiments for two years at two sites with different soil and climate conditions, which helped to understand key functional processes and the degree of generality of species-mixtures related to S uptake.

2. Materials and Methods

2.1. Experimental design and cover crop management

Four field experiments were conducted in two years (2014 and 2015) at two sites: 1) the Lamothe experimental farm of INP-EI Purpan, located in Seysses, 20 km south of Toulouse, south-western France (43.506° N, 1.237° E) on a silt clay loam soil; and 2) the La Vannelière research station of Jouffray Drillaud, located 50 km south-east of Orléans, central France (47.776° N, 2.098° E) on a sandy loam soil. Experiments conducted in 2014 and 2015 at Lamothe (L) are referred to as L2014 and L2015, respectively, and at La Vannelière (V) as V2014 and V2015, respectively. According to the Köppen climate classification, Lamothe (Toulouse) and La Vannelière (Orléans) have an oceanic climate (temperate without dry season and with warm summers) (Table 12). The experiment at each site in each year followed a completely randomised design with three replicates in blocks. The surface area of the elementary plot, containing 10 rows for each treatment, was 18.0 m^2 for L2014 and V2014, 22.5 m^2 for L2015 and 20.0 m^2 for V2015. To avoid confusing effects of plant-plant interactions between adjacent treatments, only six rows in the middle of each plot were harvested and used for soil measurements.

Cover crop species were selected for their ability to grow rapidly during a short fallow period in the autumn. Crucifer and legume species and cultivars were selected for their diversity in shoot and root architecture, sensitivity to photoperiod and precocity. All mixtures (Table 8) contained one crucifer and one legume and were designed to minimise competition according to expert knowledge and recently published information (Tribouillois et al., 2016a). The

following eight crucifers or Brassicaceae species were used: rape (*Brassica napus* L.), white mustard (*Sinapis alba* L.), Indian mustard (*Brassica juncea* L. Czern.), Ethiopian mustard (*Brassica carinata* A.Braun.), turnip (*Brassica rapa* L. subsp. *rapa*), turnip rape (*Brassica rapa* L. subsp. *oleifera*), radish (*Raphanus sativus* L.) and rocket (*Eruca sativa* Mill.). The following nine legumes species were used: Egyptian clover (*Trifolium alexandrium* L.), crimson clover (*Trifolium incarnatum* L.), common vetch (*Vicia sativa* L.), purple vetch (*Vicia benghalensis* L.), hairy vetch (*Vicia villosa* Roth.), pea (*Pisum sativum* L.), soya bean (*Glycine max* (L.) Merr.), faba bean (*Vicia faba* L.) and white lupin (*Lupinus angustifolius* L.). As indicated, not all combinations of the crucifers and legumes were tested, but only those assumed to be highly complementary in mixtures, based on expert knowledge and previous results (e.g. Tribouillois et al., 2016), and according to the human and financial resources available.

Table 12. Management, soil and climate conditions of the four experimental sites. Climate data are sums or means of growing season measurements from the sowing date to the date of cover crop termination (biomass harvest date). L2014 and L2015 are experiments at Lamothe in 2014 and 2015, respectively. V2014 and V2015 are experiments at La Vannelière in 2014 and 2015, respectively.

Characteristic	**L2014**	**V2014**	**L2015**	**V2015**
Sowing date	19 August	31 August	24 August	22 August
Biomass harvest date	4 November	18 November	3 November	26 October
Preceding crops[a]	Fallow (3 years)	Winter barley Winter barley Rape	Winter wheat sunflower winter wheat	Winter barley rape winter barley
Soil texture	Silty clay loam	Loam	Silty clay loam	Sandy loam
Soil organic matter (0-30 cm) (g kg^{-1})	19	26	19	18
Soil mineral nitrogen at sowing (kg N ha^{-1})[b]	93	139	48	79
Soil P_2O_5 (0-30 cm) (mg kg^{-1})[c]	34	320	30	129
Soil K_2O (0-30 cm) (mg kg^{-1})[c]	120	180	120	89
Soil MgO (0-30 cm) (mg kg^{-1})[c]	420	59	410	80
Soil CaO (0-30 cm) (g kg^{-1})[c]	3.7	1.7	3.8	0.9
Cumulative irrigation (mm)	30	16	15	0
Cumulative rainfall (mm)	140	250	90	174
Daily mean temperature (°C)	19.0	14.0	18.2	12.4

[a] The first crop mentioned is the preceding crop

[b] Soil mineral nitrogen (SMN) was measured at a depth of 0-90 cm for L2014 and L2015 and 0-60 cm for V2014 and V2015.

[c] P was measured with Joret-Hébert method while K, Mg and Ca was measured with ICP AES dosage.

Species grown as sole cover crops were sown at densities recommended for cover crops and to obtain plant cover with similar densities and rapid growth and soil coverage. Sowing densities for sole cover crops were 800 seeds m^{-2} for all clover species; 150 plants m^{-2} for Ethiopian mustard; 100 plants m^{-2} for white mustard; Indian mustard, rocket and all vetch species; 80 plants m^{-2} for rape, radish, turnip rape, turnip and pea; 70 plants m^{-2} for soya bean and white lupin; and 40 plants m^{-2} for faba bean. Sowing densities for mixtures were half of the corresponding sole cover crop density of each species (50% crucifers:50% legumes) to create a substitutive design of species mixtures. Seeds of both species were mixed in the row and sown with an experimental seeding machine, similar to a drill-seeder, to obtain a homogenous mixed-plant cover crop in the row to maximise plant-plant interactions.

After shallow tillage, seeds were sown 1.5-2.0 cm deep in rows 15 cm apart. The percentage of each sown species was monitored during crop emergence. Irrigation was applied to L2014, L2015 and V2014 after sowing to ensure homogeneous emergence and establishment of cover crops. No irrigation was applied to V2015 because rainfall was sufficient for plant emergence. Irrigation and fertilisation were not applied during the cover crop growing period to mimic "normal farming conditions". Nitrogen and sulphur mineral fertilisation was applied to the previous cash crop in V2014 (89 kg N ha^{-1}, 62 kg $S0_3$ ha^{-1}) and V2015 (120 kg N ha^{-1}, 69 kg $S0_3$ ha^{-1}) while no S fertilisation was applied to the previous cash crop in L2015 (145 kg N ha^{-1}). Nothing was applied to the previous fallow in L2014. Historically no sulphur deficiency was recorded in the rotation in each site×year, neither for rapeseed when cropped. We then hypothesise that sulphur was not a limiting factor in our experiments in particular for cover crops having a shorter growing period and then a lower biomass production that leads to lower S requirements. Herbicide was sprayed to control weeds at L2014 and L2015 in September due to strong weed emergence; no herbicides were applied at V2014 and V2015, which corresponded to normal farming conditions.

2.2. Plant sampling

Cover crops were sampled 2.5-3.0 months after sowing (Table 12), which is consistent with the usual practice of incorporating cover crops into the soil before sowing the subsequent winter crop. All above-ground biomass and, for crucifers alone, root biomass, was collected from 1 m^2 per replicate. Roots were collected to a depth of ca. 30 cm by digging with a fork. Shoots and roots were separated using a sharp knife or secateurs and the root systems were carefully washed with cold water to remove remaining soil. Samples were washed, dried at

80°C for 48 h, weighed and ground to measure total C, N, and S via elemental analyses based on the Dumas method (Elementar MicroVario Cube, Germany). The clay and loam soils were too wet on sampling dates to allow us to properly sample the large proportion of fine legume roots. Thus, to estimate N and S contents in legume roots, data for shoot dry matter, the shoot:root ratio and root N and S concentrations measured in multiple legume species were obtained from an experiment conducted at the INRA Toulouse experimental site in 2012 (see Tribouillois et al. (2015) for experimental details). Based on these data, we calculated three coefficients ("k") to estimate the S uptake, C:S ratio and N:S ratio of the whole legume plant based on data for shoots alone (Table 14):

k_S_R = [S uptake of the whole plant] / [S uptake of the shoots].

k_CS_R = [C:S ratio of the whole plant] / [C:S ratio of the shoots].

k_NS_R = [N:S ratio of the whole plant] / [N:S ratio of the shoots].

2.3. Indicators used to characterise mixture performances

We used the Land Equivalent Ratio (LER) indicator to assess the complementarity and competition of crucifer and legume species for resource capture and their performances relative to those of sole cover crops. LER, defined as the sole crop area required to reach the same biomass as a multi-species mixture (Willey, 1979), is the sum of the partial LER (LERp) of each mixed species. We used the LER to assess S uptake in the same way that Bedoussac and Justes (2011) did for wheat-pea mixtures:

$$LER = LERpC + LERpL \quad \text{(Eq. 1)}$$

$$LERp = S_{upMIX} / S_{upSC} \quad \text{(Eq. 2)}$$

where LERpC and LERpL are the partial LER of crucifers and legumes in the mixtures, respectively, S_{upMIX} is the S uptake of the given species (crucifer or legume) in the mixture, and S_{upSC} is the S uptake of the same species as a sole cover crop.

LER>1 indicates that the bispecific mixture used resources more efficiently than the species grown as a sole cover crop. In contrast, LER<1 indicates that competition exceeded complementarity for resource capture, while LER=1 indicates that the balance of plant interactions is null. In a substitutive design (50% of each sole cover crop species), LERp>0.5 indicates that, at the plant level, the species takes up more S in a mixture than as a sole cover

crop, while LERp<0.5 indicates that interactions in the mixture negatively affect the species' S capture.

The potential S mineralised for the subsequent main cash crop within 6 months (S_{min}) was estimated using the equation of Eriksen et al. (2004) for cover crop species with a C:S ratio of 43-329:

$$S_{min} = S_{up} \times S_{\%ava} \qquad \text{(Eq. 3)}$$

where $S_{\%ava}$ = [70 – 0.16 × C:S ratio] / 1000, which corresponds to the percentage of S uptake (S_{up}) by cover crops (shoots + roots) that is mineralised and available for the subsequent main cash crop. Mineralised S corresponds to the S green-manure service the cover crop provides after its incorporation into the soil.

2.4. Statistical analysis

Analysis of variance (ANOVA) was used to assess effects of site, year and cover crop type (sole cover crop or mixture) on three variables: 1) S uptake (shoots + roots), 2) C:S ratio and 3) mineralised S. Tukey's post-hoc test was used to distinguish differences among cover crop types for each site×year. Normality was tested using the Shapiro-Wilk test. Nonparametric Wilcoxon tests were performed to test 1) differences between cultivars of individual species for each of the three variables analysed and 2) the significance of differences in LER between cover crops and their relative difference from the value of 1.0, as well as those between LERpC and LERpL and 0.5. Statistical analyses were performed using R software (R Core Team, 2016), and differences among treatments were considered significant at P<0.05.

3. Results

3.1. Statistical analysis

ANOVA indicated that site, year and cover crop type (sole cover crop or mixture) significantly influenced S uptake and the estimated amount of S mineralised (Table 13). Site × year interactions were significant only for S uptake, while site × cover crop type interactions were significant for both variables. Interactions between year × cover crop type and among site × year × cover crop type were not significant. The nonparametric Wilcoxon test indicated that cultivars of the same species had few significant differences in performance as either sole cover crops or mixtures. The only significant difference occurred for sole cover crops of Ethiopian mustard at L2014, in which cv. *Carbon* had lower S uptake

(P=0.02) and mineralised S (P=0.02) than cv. *Utopia*. Consequently, we considered cultivars as replicates of their species to obtain more robust and generic conclusions about performances of species mixtures vs. sole cover crops. This approach does not suggest that cultivar variability is not of interest, but under our conditions it was not the main factor that influenced the most notable results.

Table 13. ANOVA probabilities for sulphur (S) uptake and estimated mineralised S (green-manure effect) influenced by experimental site (Lamothe or la Vannelière), year (2014 or 2015) and cover crop type (crucifer sole cover crops, legume sole cover crops, or mixtures) as fixed effects.

Fixed effect	S uptake	Mineralised S
Site	*	***
Year	**	*
Cover crop type	***	***
Site × year	**	ns
Site × cover crop type	**	**
Year × cover crop type	ns	ns
Site × year × cover crop type	ns	ns

3.2. Importance of including crucifer roots when estimating biomass and S uptake

The percentage of S uptake contained in roots among the four experiments ranged from 9% (rocket) to 45% (radish) for crucifers and from 6% (purple vetch) to 37% (fava bean) for legumes (Table 14). The coefficient (k_S_R), used to estimate S uptake by the whole plant ranged from 0.95-1.32 for crucifers and 0.74-1.02 for legumes. Unlike that of crucifers, the C:S ratio of legumes was lower in roots than in shoots, indicating that S was partitioned differently within the plant. These two coefficients demonstrated the importance of including roots when estimating total S uptake and the C:S ratio of the whole plant. This enables an accurate estimate of S uptake and, consequently, the S mineralised from cover crop residues which becomes available for the subsequent main cash crop. Also, k_NS_R of crucifers and legumes was less than 1, which indicates that the N:S ratio was lower in roots than in shoots of both types of species. For consistency, the results that follow are presented for the whole plant (shoots + roots) for all variables analysed.

Table 14. Carbon (C), nitrogen (N) and sulphur (S) indicators for shoot and root allocation of cover crops at the termination of crucifer and legume species. Values correspond to the mean of the four experimental years for crops grown as sole cover crops and in mixtures. S_R:S_T is the proportion of total S captured by the whole plant (shoots + roots) found in the roots. C_T:S_T is the C:S ratio of the whole plant. N_T:S_T is the N:S ratio of the whole plant. Coefficient k is used to estimate the S uptake, C:S ratio or N:S ratio of the whole plant when only those of the shoots are known. k_S_R = S uptake of the whole plant / S uptake of the shoots. k_CS_R = C:S ratio of the whole plant / C:S ratio of the shoots. Values in brackets represent standard error. k_NS_R = N:S ratio of the whole plant / N:S ratio of the shoots. Values in brackets represent standard error.

Species	**S_R:S_T**	**k_S_R**	**C_T:S_T**	**k_CS_R**	**N_T:S_T**	**k_NS_R**
rape	0.22 (0.03)	1.16 (0.01)	103 (5)	1.06 (0.01)	5.0 (0.3)	0.92 (0.01)
white mustard	0.16 (0.02)	1.16 (0.01)	109 (7)	1.05 (0.02)	4.8 (0.2)	0.92 (0.01)
Indian mustard	0.24 (0.02)	1.32 (0.04)	66 (2)	1.15 (0.05)	3.6 (0.2)	0.92 (0.01)
Ethiopian mustard	0.15 (0.02)	1.13 (0.01)	88 (4)	1.21 (0.03)	3.7 (0.2)	0.95 (0.01)
turnip	0.37 (0.02)	1.58 (0.05)	82 (4)	1.32 (0.04)	3.5 (0.3)	1.03 (0.05)
turnip rape	0.36 (0.03)	1.56 (0.08)	81 (6)	1.07 (0.03)	4.2 (0.3)	0.91 (0.02)
radish	0.45 (0.03)	1.80 (0.07)	85 (3)	0.95 (0.03)	4.2 (0.2)	0.81 (0.02)
rocket	0.09 (0.01)	1.10 (0.01)	48 (1)	1.00 (0.01)	3.3 (0.3)	0.99 (0.01)
Mean of crucifers	***0.25 (0.01)***	***1.35 (0.01)***	***83 (1)***	***1.07 (0.01)***	***4.0 (0.1)***	***0.93 (0.01)***
Egyptian clover	0.08 (0.01)	1.08 (0.01)	305 (18)	1.02 (0.00)	18.8 (0.4)	0.97 (0.01)
crimson clover	0.11 (0.01)	1.13 (0.02)	229 (7)	0.94 (0.01)	18.9 (0.7)	0.93 (0.01)
common vetch	0.14 (0.01)	1.16 (0.02)	197 (5)	0.91 (0.01)	19.1 (0.6)	0.91 (0.02)
purple vetch	0.06 (0.01)	1.06 (0.01)	209 (8)	0.97 (0.00)	18.8 (0.4)	0.96 (0.01)
hairy vetch	0.08 (0.01)	1.08 (0.01)	203 (8)	0.95 (0.01)	17.2 (0.5)	0.95 (0.02)
pea	0.08 (0.01)	1.09 (0.01)	162 (14)	0.95 (0.01)	15.2 (0.5)	0.95 (0.01)
soya bean[a]	0.19 (0.01)	1.24 (0.02)	344 (30)	0.89 (0.02)	15.8 (0.2)	0.88 (0.02)
fava bean	0.30 (0.01)	1.43 (0.02)	206 (16)	0.84 (0.02)	14.2 (0.2)	0.81 (0.01)
lupin	0.37 (0.02)	1.58 (0.02)	173 (2)	0.72 (0.02)	18.4 (1.4)	0.74 (0.02)
Mean of legumes	***0.16 (0.01)***	***1.21 (0.01)***	***225 (3)***	***0.91 (0.01)***	***17.5 (0.2)***	***0.90 (0.01)***

[a] Because soya bean were not inoculated with *Rhizobium japonicum*, they did not fix N_2.

3.3. Potential S catch-crop service

Mean S uptake of crucifer sole cover crops was 12.1 kg S ha^{-1} and ranged from 7.2 kg S ha^{-1} for rape at L2015 to 23.0 kg S ha^{-1} for radish at L2014 (Figure 16A). As a sole cover crop and overall, radish captured the most S, with a mean of 16.0 kg S ha^{-1}. Mean S uptake of legume sole cover crops was 5.0 kg S ha^{-1} and ranged from 0.6 kg S ha^{-1} for soya bean – which did not grow well due to a complete lack of N_2 fixation – at V2014 to 3.4 kg S ha^{-1} for common vetch at V2014 (Figure 16B). No significant difference in S uptake was observed among years and sites for legume species in sole cover crops.

Mean S uptake of mixtures was 12.0 kg S ha^{-1} and more similar among mixtures than it had been among crucifer sole cover crops, ranging from 8.1 kg S ha^{-1} for mixtures including rape

at L2015 to 16.1 kg S ha^{-1} for mixtures including radish at L2014 (Figure 16A). Among years and sites, no significant difference in S uptake was observed for a given crucifer species or legume species in the mixture. Furthermore, we did not observed any differences in growth stages for crucifers and legumes species in mixtures compared to sole cover crops.

For each site × year, mean S uptake of all mixtures did not differ significantly from that of all crucifer sole cover crops, but was significantly higher than that of all legume sole cover crops (Figure 17A). Mean S uptake of mixtures did not differ significantly from that of their corresponding crucifer sole cover crops either, except for mixtures including radish ($p<0.001$) and rocket ($p<0.01$), which reached 84% and 66% of the S uptake of their sole cover crops, respectively (Figure 16A). In contrast, mean S uptake of mixtures was significantly higher than that of all of their corresponding legume sole cover crops (Figure 16B).

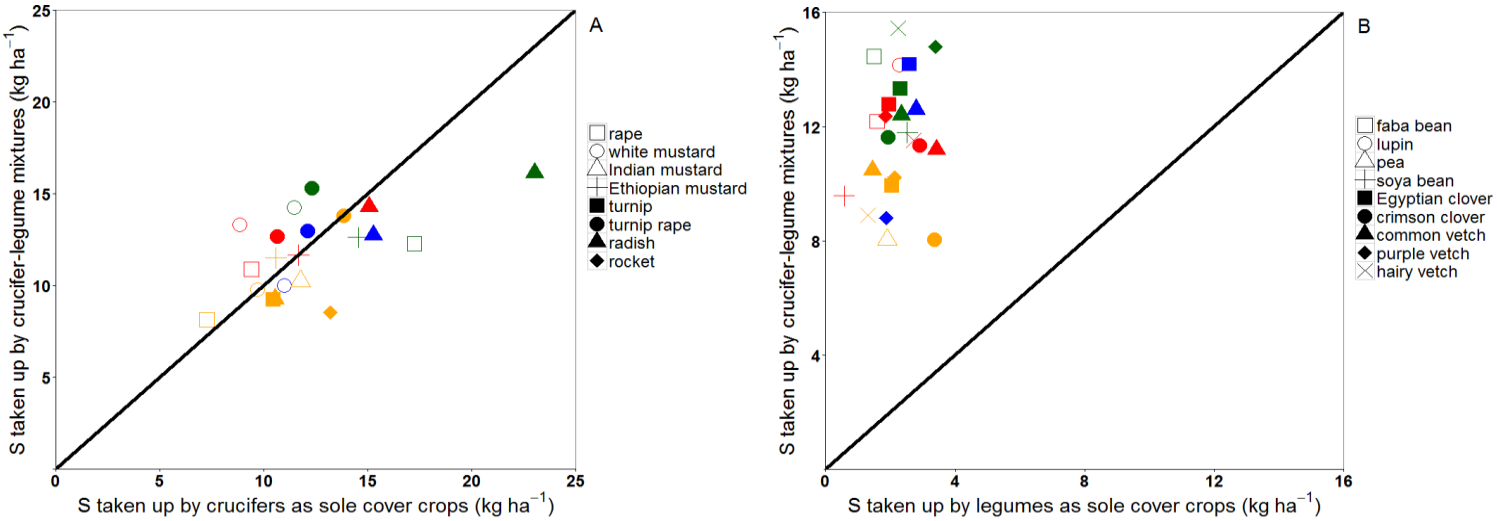

Figure 16. Total S taken up (kg ha^{-1}) by mixtures (crucifer + legume) as a function of S taken up by the corresponding (A) crucifer sole cover crop and (B) legume sole cover crop. Each point represents the mean of species cropped as sole cover crops (x-axis) or the mean of mixtures including the species (y-axis). The sold line represents y=x. Dashed lines represent y=1.5y and y=0.5x. Each point represents the mean of mixtures. Colours refer to the experimental sites La Vannelière (V) and Lamothe (L) in 2014 and 2015: V2014 in red, V2015 in blue, L2014 in green, and L2015 in yellow.

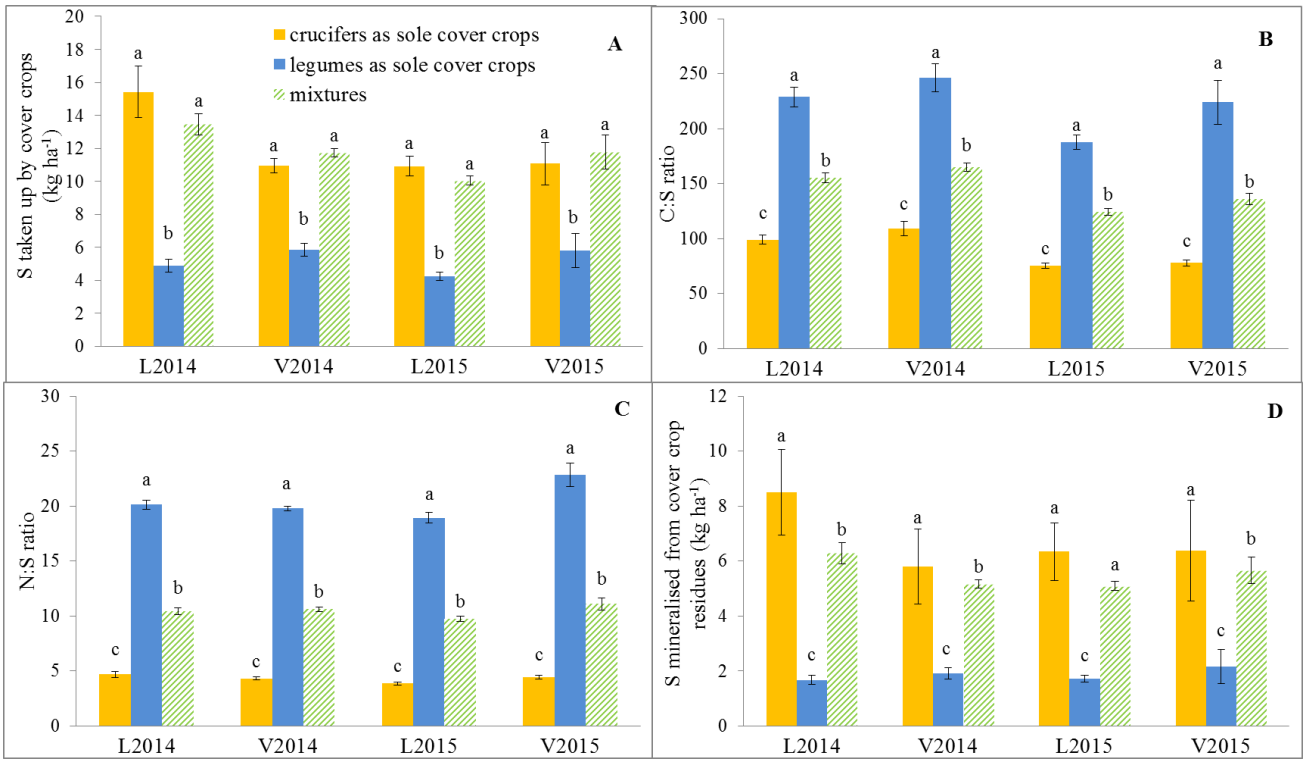

Figure 17. Mean cover crop performances measured among all treatments by site and year for crucifers and legumes in sole cover crops and mixtures on the termination date: A) sulphur (S) taken up by the whole plant; B) carbon (C):S ratio of the whole plant; C) nitrogen (N):S ratio of the whole plant; and, D) estimated S mineralised from cover crop residues after termination, corresponding to the S green-manure service. Experimental sites were La Vannelière (V) and Lamothe (L) in 2014 and 2015. Letters (a,b,c) indicate treatments with significant differences at $P<0.05$. Error bars represent standard errors.

3.4. Plant-plant interactions in mixtures: complementarity and competition between species

Mean total LER among all experiments was 1.27 and was significantly greater than 1, ranging from 1.20 at L2015 to 1.35 at V2014 (Table 15), indicating that these bispecific mixtures captured more S than their corresponding sole cover crops in the four conditions tested (2 sites × 2 years). Total LER was significantly greater than 1 for all mixtures except the one including rocket (Figure 18). Mixtures including radish (1.10) and rocket (1.00) had lower LER than those including other crucifers, with a significant difference only for turnip rape, which had the highest LER (1.38) (Figure 18).

Mean partial LER of crucifers (LERpC) among all experiments was 0.75 and was significantly greater than 0.5, ranging from 0.70 at V2015 to 0.84 at V2014 (Table 15), indicating that crucifer species captured more S in mixtures than as sole cover crops. Mean LERpC was significantly greater than 0.5 each crucifer species except rocket (0.46) (Figure 18). Mean partial LER of legumes (LERpL) among all experiments was 0.51 and did not

differ significantly from 0.5, ranging from 0.45 at L2015 to 0.57 at L2014 (Table 15), indicates that legume S uptake tended not to change in mixtures. Nevertheless, legume competition for S depended on the crucifer used in the mixture (Figure 18). Indeed, LERpL was significantly lower than 0.5 in mixtures with radish (0.37) and turnip rape (0.40), significantly greater than 0.5 in mixtures with rape (0.63), and did not differ significantly from 0.5 in mixtures with rocket (0.54), turnip (0.51), white mustard (0.50) and Ethiopian mustard (0.50).

Table 15. Mean partial Land Equivalent Ratio (LER) of crucifers (LERpC), legumes (LERpL) and LER for sulphur (S) uptake measured for all cover crops. Each value represents the mean of species mixtures for experiments conducted Lamothe (L) and La Vannelière (V) in 2014 and 2015. Letters (a, b) indicate homogeneous groups tested within columns for the four site × year treatments at P<0.05. Asterisks indicate that LERp and LER are significantly greater than 0.5 and 1.0, respectively. Values in brackets represent standard error.

Site	LERpC	LERpL	LER
L2014	0.72 (0.08) a*	0.57 (0.06) a	1.30 (0.03) a*
V2014	0.84 (0.09) b*	0.51 (0.06) a	1.35 (0.02) a*
L2015	0.75 (0.07) a*	0.45 (0.04) a	1.20 (0.02) a*
V2015	0.70 (0.13) ab*	0.53 (0.09) a	1.24 (0.03) a*
Mean	0.75 (0.09) *	0.51 (0.06)	1.27 (0.02) *

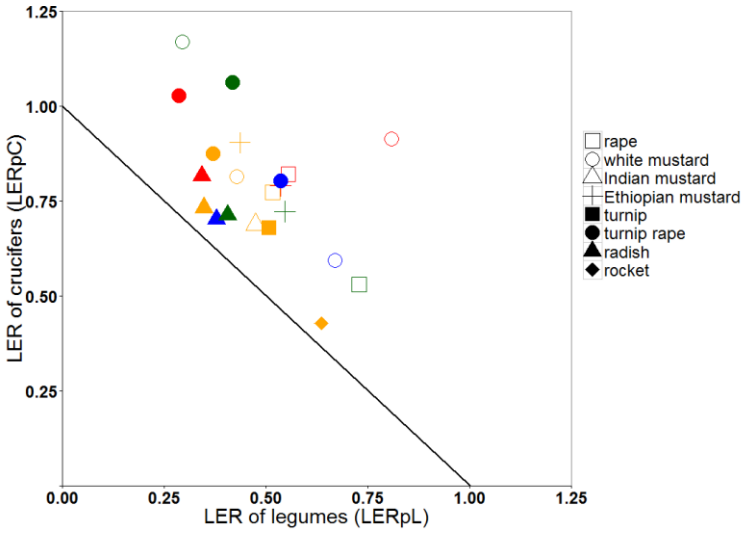

Figure 18. Partial Land Equivalent Ratios (LER) for sulphur (S) uptake of crucifers (LERpC) as a function of that of legumes (LERpL) according to (A) crucifer species and (B) legume species. Each point represents the mean of species cropped in mixtures. The solid line represents y=x. Dashed lines represent LERpC=0.5 and LERpL=0.5. Colours refer to the experimental sites La Vannelière (V) and Lamothe (L) in 2014 and 2015: V2014 in red, V2015 in blue, L2014 in green, and L2015 in yellow.

3.5 Estimated S green-manure service

The mean C:S ratio of crucifer sole cover crops was 83, ranging from 48 (rocket) to 109 (white mustard). The mean C:S of legume sole cover crops was higher (225), ranging from 173 (white lupin) to 344 (soya bean) (Table 14). The mean C:S ratio of mixtures was 145, significantly lower than that of legume sole cover crops but significantly higher than that of crucifer sole cover crops (Figure 19B). Crucifers in mixtures had a significantly lower mean

C:S ratio than they did as sole cover crops ($p<0.001$) (Figure 19A), indicating that crucifers contained more S when cropped with legumes. Legumes in mixtures did not have a significantly different C:S ratio than they did as sole cover crops, indicating that their S content did not change significantly when cropped with crucifers ($p<0.001$) (Figure 19B).

The mean amount of S estimated to have mineralised from crucifer sole cover crops was 6.8 kg S ha^{-1} and ranged from 4 kg S ha^{-1} for white mustard at L2015 to 13.0 kg S ha^{-1} for radish at L2014 (Figure 20). Among crucifer species in sole cover crops, radish had a mean S green-manure service (9.1 kg S ha^{-1}) significantly higher than that of other crucifers. Among legume species in sole cover crops, the mean S green-manure service was 1.9 kg S ha^{-1} and did not differ significantly among species. Among mixtures, the mean S green-manure service was 5.6 kg S ha^{-1} and ranged from 3.9 kg S ha^{-1} for rocket mixtures (L2015) to 7.8 kg S ha^{-1} for turnip rape mixtures (L2014). Compared to sole cover crops, crucifer species in mixtures had more similar S green-manure services; no significant differences were observed among them (Figure 20).

The mean S green-manure service of mixtures reached 85% of that of crucifer sole cover crops and was significantly greater than that of legume sole cover crops in all experiments (Figure 19D). Among all sites, mixtures had significantly lower mean S green-manure services than their corresponding crucifer sole cover crops, except for white mustard and turnip rape. Mixtures including Ethiopian mustard provided 85% of the S green-manure service provided by the same crucifer species as a sole cover crop; this value was 80% for rape, 76% for Indian mustard, 73% for radish, and 48% for rocket (Figure 20).

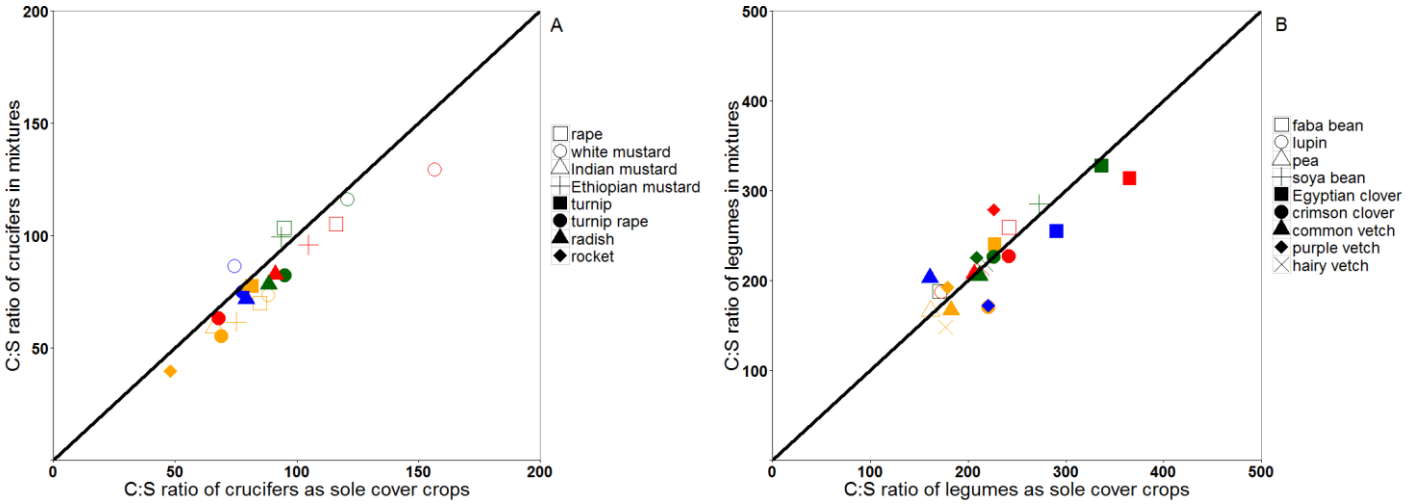

Figure 19. C:S (shoots + root parts) of crucifers (A) and legumes (B) in mixtures as a function of C:S for the corresponding crucifer and legume sole cover crop. Each point represents the mean of species cropped as sole cover crops (x-axis) or in mixtures (y-axis). The sold line represents y=x. Dashed lines represent y=1.5y and y=0.5x. Colours refer to the experimental sites La Vannelière (V) and Lamothe (L) in 2014 and 2015: V2014 in red, V2015 in blue, L2014 in green, and L2015 in yellow.

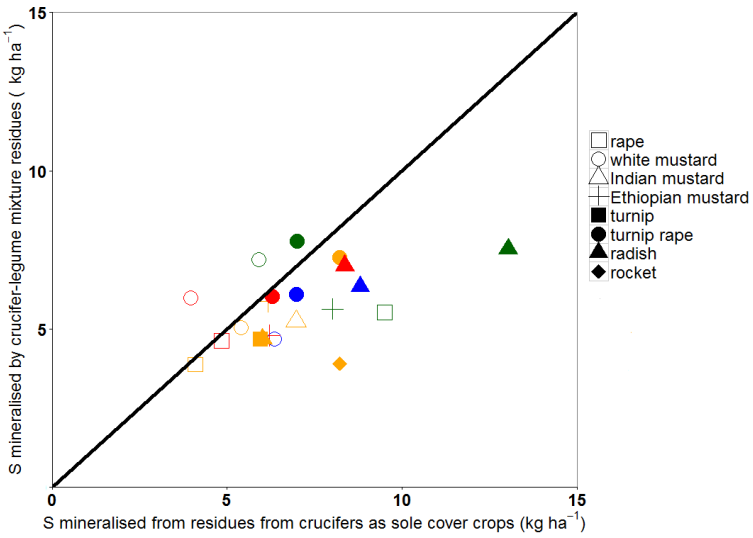

Figure 20. Mineralised S estimated from cover crop residues (kg ha^{-1}) within 6 months by mixtures (crucifer + legume) as a function of S mineralised by the corresponding crucifer sole cover crop. Full line represents y=x. Dashed line represents respectively y=1.5y and y=0.5x. Each point represents the mean of species cropped in sole cover crops (x-axis) or the mean of mixtures with the corresponding species (y-axis). Colours refer to the experimental sites La Vannelière (V) and Lamothe (L) in 2014 and 2015: V2014 in red, V2015 in blue, L2014 in green, and L2015 in yellow.

4. Discussion

4.1. Crucifer-legume mixtures provide the same sulphate catch-crop service that of crucifer sole cover crops

Bispecific crucifer-legume mixtures had the same potential to provide the S catch-crop service as crucifer sole cover crops. This highlights complementarity between crucifers and legumes in S uptake from the soil. This complementarity occurred despite the wide variety of plant architecture and precocity of the associated species, except for rocket, which was dominated by its legume companions. This complementarity also occurred in diverse sites with varying soil N availability and presumably without S limiting conditions. This could be due to 1) niche complementarity in nutrient capture (e.g. Couëdel et al., 2018; Tribouillois et al., 2016), inducing deeper and faster root growth in both component species (Li et al., 2006; Tosti and Thorup-Kristensen, 2010), and 2) acquisition of other nutrients, such as P or micronutrients, which promote root growth and rhizosphere functioning. The similar S uptake of mixtures and crucifer sole cover crops may also have been due to the density of the latter being too high, which may have induced too much intra-specific competition and decreased S availability in the soil. More intra-specific competition for crucifers as sole cover crops than in mixtures (sown at half the density) could have decreased S uptake per plant. Our study also confirms the significant S catch-crop service provided by a wide variety of legume sole cover crops which, by taking up large amounts of SMS, mitigate potential sulphate leaching during winter, as widely demonstrated in the literature for nitrate (e.g. Tonitto et al., 2006; Tribouillois et al., 2016). Thus, growing a legume sole cover crop would always be preferable to leaving soil bare, thereby decreasing nutrient losses, recycling nutrients (N, S and probably

other nutrients), and improving soil chemical fertility by adding C to the soil by producing biomass (C sequestration service).

Among species, radish provided a higher S catch-crop service as a sole cover crop than rape, as also shown by Eriksen and Thorup-Kristensen (2002), and then seven other crucifer species. When mixed with legumes, crucifers had no significant differences in S uptake, except for rocket, which took up less S due to less growth during the juvenile phase. As the S uptake of most crucifers did not differ greatly under our conditions, the crucifer species could be chosen based on other properties or targeted services, such as biofumigation potential (Matthiessen and Kirkegaard, 2006) or soil structure improvement (Blanco-Canqui et al., 2015). Nevertheless, changing species sown as cover crops each year in a given rotation is recommended to avoid pathogen specialisation. Our study indicates that a wide variety of crucifers could be combined with legume species in cover crop mixtures, facilitating the design of agroecological cropping systems that are effective from multiple viewpoints and that provide multiple ecosystem services.

4.2. Crucifer-legume mixtures can provide a sulphur green-manure service similar to that of crucifer sole cover crops

Our study assessed the potential S green-manure service of cover crops by considering all the S taken up by and contained in shoots and roots, which is a surprisingly new approach according to our analysis of the scientific literature. Our results highlighted that crucifer-legume mixtures can provide a mean level of S green-manure service (85%) similar to that of crucifer sole cover crops, for at least three reasons: 1) species mixtures and crucifer sole cover crops took up equivalent amounts of S and 2) crucifers took up a large proportion of S taken up by mixtures, which gave mixtures 3) an intermediate C:S ratio. We also observed a lower C:S ratio per crucifer plants when they were in mixtures, probably due to complementarity in S acquisition with legumes.

Among species, radish and rape provide a high S green-manure service, as Eriksen and Thorup-Kristensen (2002) demonstrated when assessing the S capture of subsequent spring barley. We demonstrated the positive S green-manure service provided by six additional crucifer species, which was similar to that of radish, increasing the number of crucifer species that can be grown in cover crops to increase S-related services. In mixtures, no difference in S uptake was found among crucifers, except for mixtures with rocket, which took up significantly less S because it produced less biomass.

Our study also demonstrated the importance of including the S content of roots of crucifers and, to a lesser extent, of legumes, to estimate S catch-crop and green-manure services more accurately. We provide new references for estimating the S content of crucifer roots, which renders estimates of S uptake and recycling services more accurate. Both root S uptake (kg S ha^{-1}) and root C:S status depended on crucifer and legume species but also varied among sites. The shoot:root ratio should then be estimated under a wider range of environmental conditions to obtain more data about it and increase the relevance of assessing the S green-manure service provided by cover crops without always needing to sample legume roots in the soil.

4.3. Crucifer-legume inter-specific competition and complementarity

Our study revealed LER values significantly greater than 1 and often reaching 1.2, indicating that crucifer-legume mixtures were effective in capturing S. Crucifers in all experiments benefited from the mixtures, having a LERpC significantly higher than the theoretical value of 0.5. Consequently, we can hypothesise that crucifers in mixtures experienced more intra-specific competition than inter-specific competition, the latter enabling significant expression of niche complementarity for resource use and/or facilitation processes. Overall, crucifers are the most effective catch crops because they grow the fastest (Thorup-Kristensen et al., 2003) and thus compete strongly to capture abiotic resources. The competition between crucifers and legumes for S uptake confirms the results of the few studies we found under field conditions for non-legume-legume cash crops, such as one which observed an LERtotal>1 for a barley-pea mixture (Andersen et al., 2007; Hauggaard-Nielsen et al., 2009). We observed that competition with legumes was greater for radish and turnip rape, which have larger roots (taproot) than other species. Radish is one of the fastest growing crucifers and rapidly takes up SMS (Thorup-Kristensen, 2003; Kristensen and Thorup-Kristensen, 2004). Our results also showed no strong incompatibilities in the bispecific mixtures, despite the high and variable competition for abiotic resources and the allelopathic effects which crucifers can generate in cover crops (Gimsing and Kirkegaard, 2009). Demonstrating allelopathic effects under field conditions, however, requires specific measurements (e.g Falquet et al., 2014). We can only hypothesise that allelopathic effects explain certain differences in results we obtained, in particular between radish or turnip rape and the three mustard species or rape, since each of the two groups is known to not produce the same types of GSL (Kirkegaard and Sarwar, 1998; Bellostas et al., 2007).

4.4. Mixtures for providing multi-service cover crops: linking S recycling and other services

S capture is closely linked to N capture since S and N are metabolised to produce protein; thus, S-related services provided by cover crops influence N-related services, and vice-versa, and both services should be considered together. S deficiency in grassland soil has been shown to restrict N uptake and thus decrease the N catch-crop service (Brown et al., 2000) and potentially the N green-manure service. S also is essential for maintaining symbiotic N_2 fixation in legumes (Scherer and Lange, 1996; Varin et al., 2010); for example, S deficiency in alfalfa decreased N_2 fixation (DeBoer and Duke, 1982), but other physiological functions remained relatively unaffected. Low S availability can result from competition for resources in species mixtures. Legumes play a key role by increasing the N green-manure service in cover crop mixtures (Tribouillois et al., 2016; Couedel et al., 2018). Competition for S uptake by high S-demanding crucifers, however, could lower the N status of legumes and decrease the N green-manure service. In a pot experiment, Génard et al. (2016; 2017) observed that legumes in a mixture with rape had a lower S content than as a sole crop, but did not observe lower N_2 fixation. In our study, the S content of legumes in mixtures tended to differ little from that as sole cover crops (i.e. no decrease in S concentration per unit of biomass). We can hypothesise that placing legumes into mixtures did not impact their N_2 fixation and thus their S and N statuses. As the mean partial LER for crucifers was 0.75 and that for legumes was almost 0.5, we can conclude that crucifers benefited from association with legumes, most likely due to better N and S statuses.

Linking the results of this study to those of Couedel et al (2018), who focused on N services provided by cover crop mixtures, demonstrates that including legumes in species mixtures tended to significantly increase the N green-manure service compared to that of crucifer sole cover crops without decreasing services associated with N and S cycles (Table 16). Thus, compared to the crucifer sole cover crops, crucifer-legume cover crops can provide the same level of catch-crop service and at least two thirds of the level of green-manure service provided by legumes sole cover crops.

The amount of S taken up by crucifers could also determine the production of S-based secondary metabolites such as GSL. The amount of GSL produced determines the potential allelopathic effect of crucifer cover crops on a wide variety of living soil organisms (Couëdel et al., 2017; Matthiessen and Kirkegaard, 2006). Low S availability could decrease the S status of crucifers and thus the production of GSL (Li et al., 2007a; Omirou et al., 2009),

especially highly toxic ones containing 3 S molecules from methionine and cysteine amino acids (Falk et al., 2007). Designing cover crop mixtures that decrease competition for crucifer S capture could be a good way to avoid a decrease in GSL production. Secondary metabolite production in plants in response to nutrient supply is complex, and further studies are required to determine the influence of species mixtures on crucifer GSL production (Couedel et al., submitted).

Table 16. Services associated with nitrogen (N) and sulphur (S) cycles for crucifer and legume in sole crop (SC) or in mixture (crucifer + legume). Data for N catch-crop and N green-manure services come from Couëdel et al. (2018). Data for S catch-crop and S green-manure services come from the current study.

Service	Mean crucifer SC	Mean legume SC	Mean mixtures
N catch crop	100%	66%	98%
N green manure	18%	100%	63%
S catch crop	100%	30%	99%
S green manure	100%	23%	85%

5. Conclusion

Bispecific crucifer-legume cover crop mixtures provided as much S catch-crop service and similar S green-manure service as crucifer sole cover crops, which are widely used by European farmers. These S catch-crop and green-manure services were validated for a wide variety of crucifer and legume species that differ in architecture, precocity and C:S ratio. The same results were also found for two sites having very different soil and N available at sowing while S was presumed to be not limiting. Both S-related ecosystem services were based on combining two species that had suitable complementarity for abiotic resource capture and use efficiency. Even though crucifers strongly competed with legumes for the main abiotic resources, no bispecific mixtures were incompatible with legume development and growth. The S status of legumes was likely high enough to avoid an S deficiency severe enough to decrease the N green-manure service. Crucifer-legume mixtures provided similar S-related ecosystem services as crucifer sole cover crops, in addition to well-known N services, and for a wide variety of species. This supports the practice of diversifying the crucifer species included in cover crops in European temperate cropping systems. An even greater potential exists to alternate species from one year to the next when designing multi-species mixtures to obtain desired ecosystem services.

Chapter 5. Crucifer glucosinolate production in legume-crucifer cover crop mixtures.

This chapter is publish as :

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Graphical representation of research objectives:

In this chapter for various cultivars of nine crucifer species we ask the questions:

- Do crucifers in mixtures have the same GSL type and concentrations than in sole crops?
- In which extent do mixtures have a lower GSL production per basis area compared to crucifer pure crop?

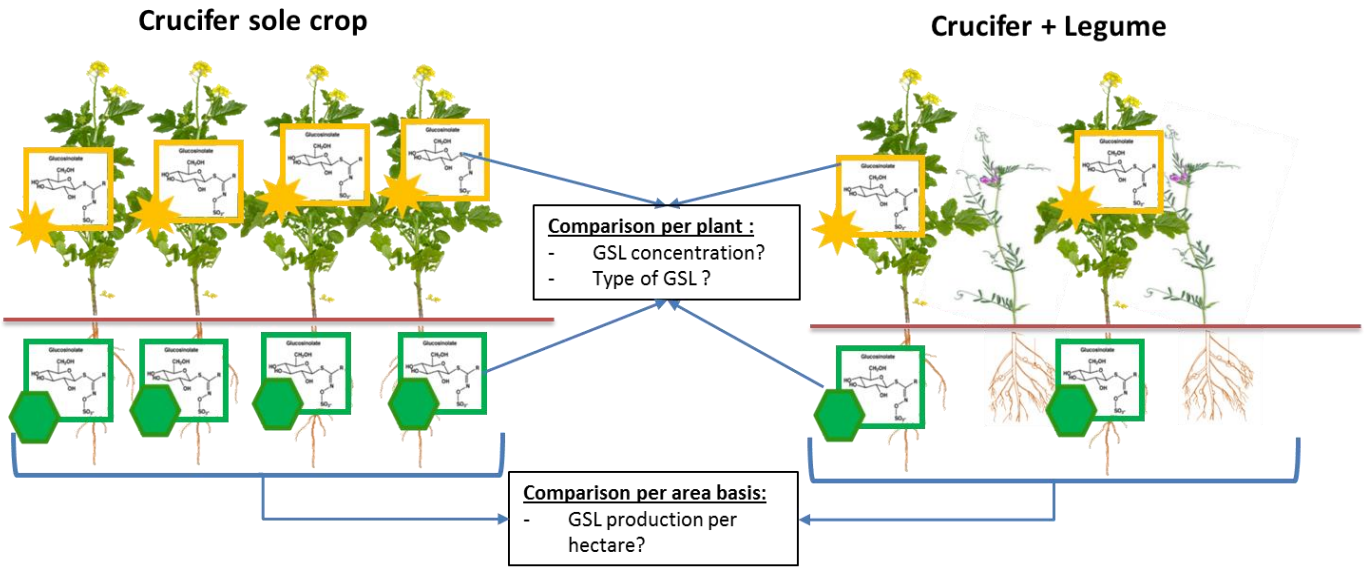

Figure 21. Summarized scheme of questions raised by the chapter 5

1. Introduction

Biodiversity based agriculture has been promoted as one tool to enhance ecosystem services while reducing disservices engendered by anthropogenic inputs (Duru et al, 2015). Cover crops grown during the autumn-winter period between two cash crops in annual crop sequences can provide a number of ecosystem-services including improved soil and water quality (Blanco-Canqui et al., 2015; Dabney et al., 2001), nutrient cycling (Thorup-Kristensen et al., 2003) and suppression of weeds (Teasdale, 1996), pests (Farooq et al., 2011) and diseases (Snapp et al., 2005). Among the species available, Brassicaceae or crucifer cover crops have an additional and unique capacity to suppress pests and diseases that is related to the bioactive hydrolysis products of the family of compounds known as glucosinolates (GSLs) (Brown and Morra, 1997; Van Dam et al., 2009). GSLs are sulfur (S) and nitrogen (N) containing beta-thioglucoside secondary metabolites present in the plant vacuole (Agerbirk and Olsen, 2012). When plant tissues are disrupted, GSLs react with endogenous enzymatic myrosinases to produce numerous biocidal compounds such as isothiocyanates, thiocyanates and nitriles (Brown and Morra, 1997). Intact GSLs released from roots into the soil environment may also be hydrolysed by microbial myrosinases to generate the same products exogenously in the rhizosphere (Gimsing and Kirkegaard, 2009). The types and concentrations of GSLs vary significantly among crucifer species, and between root and shoot tissues of the same species (Bellostas et al., 2007; Kirkegaard and Sarwar, 1998) and the ecological and agronomic implications of GSL interactions with pathogens have been considered in several reviews (Brown and Morra, 1997; Van Dam et al., 2009). The interaction of crucifer cover-crop derived GSLs with living organisms in the environment can occur 1) during the crucifer cover crop growing period when GSL and hydrolysis products are permanently released in the rhizosphere (McCully et al., 2008; Rumberger and Marschner, 2004)**,** but also 2) when the cover crop is terminated and the biomass incorporated as either brown (killed by herbicide) or green manure. Evidence for biocidal effects both during growth and after termination of cover crops have been demonstrated (Motisi et al., 2009a, 2010) although much higher concentrations of GSLs and hydrolysis products from both shoot and root tissues have been measured following incorporation (Gimsing and Kirkegaard, 2009). The suppression of pests and pathogens associated with these compounds has been termed “biofumigation” (Matthiessen and Kirkegaard, 2006) and has generated interest from an ecological and agronomic perspective in the regulation of soil biological communities (Bressan et al., 2013), including on

nematodes (Potter et al., 1998), fungi (Motisi et al, 2009a), bacteria (Arthy et al., 2002) and weeds (Haramoto and Gallandt, 2004; Jabran et al., 2015). Strategies to capture the beneficial effects of pathogen suppression without negative impacts on beneficial or non-target organisms has been an area of recent interest (Hossain et al., 2015; Omirou et al., 2011).

The biofumigation potential of crucifer cover crops depends significantly on the GSL types and concentration in the plant (Matthiessen and Kirkegaard, 2006) which not only differs between species, but also with environment and agronomic practices (Björkman et al., 2011). Typically crucifer cover crops are grown as pure/sole crops (SC) with the aim of maximizing the biomass and GSL concentration and thereby the GSL production to enhance biofumigation potential. Recently there has been a greater focus on legume and non-legume cover-crop mixtures to enhance the multiple eco-services that may arise from ecological interactions (Finney et al., 2016; Justes et al., 2012). Often when deciding a strategy for cover crops, the highest priority is given to enhanced N-cycling eco-services, either by provision of fixed N or the capture and storage of potentially leachable N. Sole cover crop crucifers have high catch crop capacity but engender lower N mineralization than sole cover crop legumes (Couëdel et al., 2018c). Crucifer-legume bispecific mixtures (i.e. in substitutive design, such as 50% density of each species of the sole crop in the mixture) have been found to improve the amount of N mineralized for the next cash crop compared to crucifer sole crops (Tribouillois et al., 2016a). Indeed legumes provide a high input of nitrogen in the agroecosystem due to the high amount of N acquired from the soil and atmosphere (Thorup-Kristensen et al., 2003). Overall non legume-legume mixtures have been shown to produce more biomass than non-legume sole crops because of the N_2 fixation of legume increasing N inputs and also due to their better resource use efficiency, due to the niche complementarity in using abiotic resources such as light, water and nutrients (Jensen, 1996). Non-legume - legume mixtures have also been demonstrated to provide better suppression of certain pests, diseases and weeds compared to sole crops (Trenbath, 1993; Boudreau, 2013).

Despite the significant interest in pest-suppressive and catch-crop capacity of crucifer cover crops, and the concurrent interest in the potential of bispecific mixtures, there have been few studies investigating the impact of inclusion in crop mixtures on GSL profiles and production (amount of GSL produced per unit of surface) of the crucifer component. A number of different plant–plant interactions in bispecific mixtures could be anticipated to influence the types and concentrations of GSL and the level of production of crucifers and thereby the biofumigation effect such as pest-suppressive eco-services of the mixtures. For example, in

mixtures with legumes, crucifer cover crops are usually in competition for water, nutrients and light due to their rapid root and shoot growth (Kristensen and Thorup-Kristensen, 2004). As a result, on an individual plant basis, crucifers have been shown to produce a higher biomass (S. E. Wortman et al., 2012), to acquire more N (Couëdel et al., 2018c; Tribouillois et al., 2016a; Wendling et al., 2017) and more S (Génard et al., 2017) when mixed with a legume crop than in a sole crop. As N and S are both essential components of GSLs, a different GSL production per crucifer plant could therefore be expected in mixtures compared to sole crops. Indeed in sole crops, an increasing N and S supply by fertilisation has been shown to generate a higher GSL production per plant in crucifers (Falk et al., 2007; Li et al., 2007b; Omirou et al., 2009). These competitive and interactive effects could influence mechanisms of GSL production (per plant and per unit of ground area cropped) and release from growing roots or shoots during cover-crop growth, and the final total GSL production available at incorporation. At the plant level, changes in GSL may influence crucifer susceptibility to pests and diseases while the biofumigation potential of the cover crop would also be influenced by changes on an area basis. In the only two studies available investigating impacts of bispecific mixtures on GSL production, Stavridou et al., (2012) found no impact on broccoli (*Brassica oleracea*) in a substitutive design mixture (50%-50% density of each of sole crop) with lettuce (*Lactuca sativa*), while Björkman et al. (2008) found that both shoot and root GSLs in cabbage (*Brassica oleracea*) decreased when mixed with clover in an additive design (100%-100%, i.e, at the same density of each of sole crop). To our knowledge there have been no studies conducted to assess the effect of crucifer-legume mixtures on GSL production in a substitutive design under cover crops grown in arable cropping systems (field conditions).

We report a study designed to assess the impacts of crucifer-legume bispecific mixtures (50%-50% substitutive design) on the GSL type, concentration and production at both the individual plant level and on a ground area basis. The capacity of crucifers to strongly compete with legumes for water, N and S acquisition prompted us to test two hypotheses:

1) Crucifers in bispecific cover crop mixtures with legumes will have the same total GSL concentration as in the sole crop

2) Bispecific mixtures will produce more than 50% of sole crop GSL on a ground-area basis due to increased biomass production per crucifer plant with no reduction in GSL concentration.

Recent studies have highlighted that climate, soil type and N availability at sowing have the greatest influence on cover crop mixture performances (Tribouillois et al., 2016). Accordingly the experiments reported here included a range of different crucifer and legume species and varieties in the mixtures and were conducted over two years at two contrasting sites from late summer to late autumn to assess the generality of species mixture interactions.

2. Materials and Methods

2.1. Experimental design and cover crop management

Four field experiments were conducted over two years (2014 and 2015) at two sites: 1) the Lamothe experimental farm of INP-EI Purpan, located in Seysses, 20 km south of Toulouse, south-western France (43.506° N, 1.237° E), and 2) the La Vannelière research station of Jouffray Drillaud, located 50 km south-east of Orléans, central France (47,776° N, 2.098° E). Experiments conducted in 2014 and 2015 are referred to as L2014 and L2015, respectively, at Lamothe (L) and as V2014 and V2015, respectively, at La Vannelière (V). According to the Köppen climate classification, Lamothe (Toulouse) and La Vannelière (Orléans) have an oceanic climate (temperate without dry season and with warm summers) (Table 17). At all four site-years, the experiment was a completely randomised design that was replicated with three replicates in blocks. Individual sub-plots were arranged with 10 rows at 15 cm spacing for each treatment, with plot area of 18 m^2 for L2014 and V2014, 22.5 m^2 for L2015 and 20.0 m^2 for V2015. To avoid plant-plant competition effects between adjacent treatments, only the six central rows in each plot were sampled for soil and plant measurements.

Table 17. Management, soil and climate conditions of the four experimental sites. Climate data are sums or means of growing season measurements from the sowing date to the date of cover crop destruction (biomass harvest date). L2014 and L2015 are experiments at Lamothe in 2014 and 2015, respectively. V2014 and V2015 are experiments at La Vannelière in 2014 and 2015, respectively.

	L2014	**V2014**	**L2015**	**V2015**
Sowing date	19 au. 2014	31 au. 2014	24 au. 2015	22 au. 2015
Biomass harvest date	4 nov. 2014	18 nov. 2014	3 nov. 2015	26 oct. 2015
Preceding crop	Fallow (3 years)	Winter barley	Common wheat	Winter barley
Soil texture	Silt Clay Loam	Loam	Silty clay loam	Sandy Loam
SMN at sowing (kg N ha^{-1})[a]	93	139	48	79
Cumulative irrigation (mm)	30	16	15	0
Cumulative rainfall (mm)	140	250	90	174
Daily mean temperature(°C)	19	14	18.2	12.4

[a] Soil mineral nitrogen (SMN) measured at a depth of 0–90 cm for L2014 and L2015 and 0–60 cm for V2014 and V2015

A range of cover crop species were selected for their ability to grow rapidly during the autumn in a short-term fallow period. Crucifer and legume species and cultivars were selected to represent a wide diversity in shoot/root architecture, growth precocity and GSL types as deeply characterised in other studies (Tribouillois et al., 2015; Kirkegaard and Sarwar 1998). All mixtures (Table 8) contained one crucifer and one legume (bispecific mixtures) and were designed to minimise unfavourable interspecific competition between species for light capture and root development based on expert local knowledge and recently published information on the phenology of the studied species (Tribouillois et al., 2016a). The species used were as follows: 1) Crucifers tested were rape (*Brassica napus*), white mustard (*Sinapis alba*), Indian mustard (*Brassica juncea*), Ethiopian mustard (*Brassica carinata*), turnip (*Brassica rapa* subsp. *rapa*), turnip rape (*Brassica rapa* subsp. *oleifera*) , radish (*Raphanus sativus*) and rocket (*Eruca sativa*); 2) Legumes tested were Egyptian clover (*Trifolium alexandrium*), crimson clover (*Trifolium incarnatum*), common vetch (*Vicia sativa)*, purple vetch (*Vicia benghalensis*), hairy vetch (*Vicia villosa)*, pea (*Pisum sativum*), soya bean (*Glycine max*), faba bean (*Vicia faba*), and white lupin (*Lupinus angustifolius*).

Species grown as sole crops were sown at densities recommended by the cover crop seed distributor and breeder (RAGT and Jouffray-Drillaud). Densities in sole crops were 800 plants/m^2 for all clover species, 150 plants/m^2 for Ethiopian mustard, 100 plants/m^2 for white mustard, Indian mustard, rocket and all vetch species, 80 plants/m^2 for rape, radish, turnip rape, turnip and pea, 70 plants/m^2 for soya bean and lupin, 40 plants/m^2 for faba bean. In the mixtures, sowing densities for each species were half of the corresponding sole crop density of each species (50% sole crop crucifer:50% sole crop legume in each mixtures) to create a substitutive design. Seeds of both species were mixed together before sowing to ensure that they were mixed in the row, and were sown with an experimental drill-seeder to obtain a homogenous mixed-plant cover crop with a row width of 15 cm and a sowing-depth ranging from 1.5 to 2 cm.

Irrigation was applied after sowing to L2014, L2015 and V2014 to ensure homogeneous emergence and establishment of cover crops. No irrigation was applied in V2015 because rainfall was sufficient for plant emergence. Irrigation was not applied during the growing period in order to mimic “normal farming conditions”, and no fertiliser was added during the experiments which is common for cover crops used for N balance management in the region. Shallow tillage was performed at both sites before sowing the cover crop. Weed control was performed in L2014 and L2015 with a herbicide sprayed in September due to strong weed

emergence at that site, but no herbicides were applied in V2014 and V2015, which corresponded to the usual cover-crop management.

2.2. Plant sampling and GSL analysis

Cover crops were harvested 2-3 months after sowing (Table 17), which is consistent with the usual practice of cover crop termination prior to sowing the subsequent winter crop. All above-ground and root biomass of crucifers, were collected from 1 m^2 in each plot. Roots were collected to a depth of ca. 30 cm by digging with a shovel. Shoots and roots were separated using a sharp knife or secateurs and the root systems were washed with cold water to remove remaining soil. The samples were washed, dried at 80°C for 48 h, and weighed to assess total biomass production. A sub-sample of 5 plants was frozen immediately and stored at -80°C before lyophilisation for GSL analysis. GSL quantitative analysis was performed in a specialised laboratory (Institute for Water and Wetland Research, Radboud University, The Netherlands) using a method described in more detail by De Graaf et al., (2015). The method uses extraction and HPLC–UV detector to identify and quantify GSLs from the samples. The technique is able to detect a wide range of the most common GSL types found in root and shoot tissues as reported in De Graaf et al., (2015).

2.3. Indicators used to characterise the performance of the mixtures

We used the Land Equivalent Ratio (LER) indicator to assess the performance of crucifer biomass production and GSL production on a ground area basis in mixtures relative to sole cover crops (SC). LER, defined as the area of a SC required to reach the same biomass as a multi-species mixture (Willey, 1979), is the sum of the partial LER (LERp) corresponding of the performance of each species in the mixture. In the study we used only LERp of crucifers for biomass and GSL production and applied them separately for shoot and root tissues. This was necessary to account for the different GSL profiles in root and shoot tissues, and the real possibility that both biomass and GSL concentration of root and shoot tissue may respond to mixtures in different ways:

$$LER_bT = Total\_biomass_{MIX}/Total\_biomass_{SC} \quad \text{(Eq. 1)}$$

$$LER_bR = Root\_biomass_{MIX}/Root\_biomass_{SC} \quad \text{(Eq. 2)}$$

$$LER_bS = Shoot\_biomass_{MIX}/Shoot\_biomass_{SC} \quad \text{(Eq. 3)}$$

$$LER_{GSL}T = Total\_GSL_{MIX}/Total\_GSL_{SC} \quad \text{(Eq. 4)}$$

$$LER_{GSL}R = Root\_GSL_{MIX}/Root\_GSL_{SC} \quad \text{(Eq. 5)}$$

$$LER_{GSL}S = Shoot\_GSL_{MIX}/Shoot\_GSL_{SC} \quad \text{(Eq. 6)}$$

where $biomass_{MIX}$ and $biomass_{SC}$ are the biomass produced by crucifers on a ground area basis in the mixture or in the sole crop respectively. GSL_{MIX} and GSL_{SC} are the GSL produced (mmol m^{-2}) by crucifers in the mixture (MIX) or in sole crop (SC) respectively. GSL production on a ground area basis (mmol m^{-2}) is the product of the biomass and GSL concentration.

In a substitutive design (50% of each SC species), LERp>0.5 indicates that, at the plant level, crucifers have produced more biomass or GSL in mixture than as a SC, while LERp<0.5 means that one or both of these parameters have been reduced in the mixture compared to sole crop. To compare crucifer's GSL concentration between mixtures and sole cover crop we used a simple ratio: $\{[GSL]_{MIX}:[GSL]_{SC}\}$ here $[GSL]_{MIX}$ and $[GSL]_{SC}$ are the GSL produced (µmol gDM^{-1}) by crucifers in the mixture (MIX) and in sole crop (SC) respectively.

Here ratios significantly > 1 indicate increased GSL concentration in the mixture, or < 1 reductions.

2.4. Statistical analysis

Nonparametric Kruskall Wallis test was used to test the effects of legumes on crucifer biomass and GSL production. Furthermore nonparametric Wilcoxon test were performed to 1) test the differences between cultivars of a same species for each variables, and 2) test the significance of differences in $\{ [GSL]_{MIX}:[GSL]_{SC}\}$ from 1.0, as well as those between LER_b and LER_{GSL} from 0.5. For all data analysis, significant differences among treatments were identified at the 0.05 probability level of significance. Statistical analyses were performed with R software (R Core Team, 2016).

3. Results

3.1. Factors impacting GSL concentration and production

3.1.1. Crucifer cultivar effects

The experiments included a number of crucifer cultivar comparisons across the different sites and it was important to determine whether significant differences were apparent between cultivars. Few differences in GSL concentration or production were observed for the different cultivars of the same crucifer species and these differences were not consistently observed among years or between the sole crop and mixtures (Table 18). The only consistent difference observed both in sole crop and mixtures was in L2014 where turnip rape cultivar *Chicon* had higher root GSL concentration (P=0.02 in sole crop and P=0.01 in mixtures) and a significantly lower root biomass (P=0.02 in sole crop and P<0.001 in mixtures) than the turnip rape cultivar *Hector*. Nevertheless, overall root GSL production of these two turnip rape cultivars was not significantly different. As a result of this rare occurrence of varietal differences we have pooled the data for cultivars of the same species for further analysis to highlight key results and messages.

Table 18. Significance of *Wilcoxon tests* comparing performances of cover crop cultivars of a same species on crucifer GSL concentration (µmol gDM^{-1}), GSL production (mmol m^2) and biomass. Names in italic are cultivars having a significantly higher performance than the other cultivar tested for the variable considered (P<0.05). Abbreviation 'ns' signifies that no significant differences have been found between cultivars for the variable considered. Empty cells ("/") indicate that the cultivar was not sown in mixtures or as sole crops.

			Rape		White mustard		Ethiopian mustard		Indian mustard		Turnip rape		Radish	
			SC	MIX	SC	MIX	SC	MIX	SC	MIX	SC	MIX	SC	MIX
L2014	Roots	biomass	ns	ns	ns	ns	/	/	ns	ns	*Hector*	*Hector*	ns	ns
		GSL concentration	ns	*Mosa*	ns	ns	/	/	ns	ns	*Chicon*	*Chicon*	ns	ns
		GSL production	ns	*Mosa*	ns	ns	/	/	ns	ns	ns	ns	ns	ns
	Shoots	biomass	ns	ns	ns	ns	/	/	ns	ns	ns	ns	ns	*Arena*
		GSL concentration	ns	ns	ns	ns	/	/	ns	ns	*Chicon*	ns	ns	*Terranova*
		GSL production	ns	ns	ns	ns	/	/	ns	ns	*Chicon*	ns	ns	ns
	Total	biomass	ns	ns	ns	ns	/	/	ns	ns	ns	ns	ns	*Arena*
		GSL concentration	ns	*Mosa*	ns	ns	/	/	ns	ns	Chicon	ns	ns	*Terranova*
		GSL production	ns	ns	ns	ns	/	/	ns	ns	ns	ns	ns	ns
L2015	Roots	biomass	/	/	ns	ns	ns	ns	ns	ns	/	/	*Nemaflex*	ns
		GSL concentration	/	/	ns	ns	ns	*Brons*	ns	ns	/	/	ns	ns
		GSL production	/	/	ns	ns	ns	ns	ns	ns	/	/	ns	ns
	Shoots	biomass	/	/	ns	*Abraham*	ns	ns	ns	ns	/	/	ns	ns
		GSL concentration	/	/	ns	ns	ns	ns	ns	ns	/	/	*Terranova*	ns
		GSL production	/	/	ns	ns	*Etamine*	ns	ns	ns	/	/	*Terranova*	ns
	Total	biomass	/	/	ns	*Abraham*	ns	ns	ns	ns	/	/	ns	ns
		GSL concentration	/	/	ns	ns	ns	ns	ns	ns	/	/	ns	ns
		GSL production	/	/	ns	ns	*Etamine*	ns	ns	ns	/	/	ns	ns

3.1.2. Legume species effect

The experiments also included a range of different legume species mixed with each crucifer across the different sites. However, as for the crucifer cultivars we found no evidence that particular legume species had a significant specific effect on the GSL concentration and production of any crucifer species in mixtures across the different experiments (data not shown). For this reason, and to simplify further analysis and discussion of the impacts of bispecific mixtures, we have pooled the results for different legumes species in mixtures with each crucifer species. As a consequence we focus the rest of the analysis and discussion on generic bispecific mixtures of a legume with a crucifer species.

3.1.3. Overall impact of mixtures on GSL profile

No effects on GSL types in either root or shoot tissues were found when crucifers were cropped in sole crop or in mixtures (Figure 22). Furthermore, the relative proportions of aliphatic, aromatic and indole GSLs in both root and shoot tissues were also unaffected in mixtures (Figure 22). For this reason we concentrate the rest of the discussion on the impacts on total GSL concentration.

Overall the types and concentrations of GSLs found in the root and shoot tissues are within the ranges found in previously reported studies for field-grown crucifer crops (Bellostas et al., 2007; Kirkegaard and Sarwar, 1998).

Aromatic GSL represented a high percentage of total root GSL for all species except for radish and rocket (Table 19). Gluconasturtiin was the main root aromatic GSL in every crucifer species, while Sinalbin was also found in high concentration in the roots of white mustard. Aliphatic GSL represent a high proportion of total root GSL for Indian mustard, Ethiopian mustard, turnip rape, radish and rocket. Indian mustard and Ethiopian mustard contained mainly Sinigrin while radish, turnip rape and rocket contained respectively Glucoraphasatin, Progoitrin and Glucoerucin. Indole GSL were present in the roots of every species at low concentration.

Aliphatic GSL represented a high percentage of total shoot GSL for Indian mustard and Ethiopian mustard due to high concentrations of Sinigrin while turnip rape had a high level of aliphatic GSL predominately Gluconapin (Table 20). Aromatic GSL represent a high proportion of total shoot GSL only for white mustard mostly Sinalbin. Indole GSL were detected in the shoots of all species expect for white mustard, and represent a high proportion

of GSL for turnip rape (mostly 4methoxyglucobrassicin), radish (Glucobrassicin) and rocket (indole 18.503).

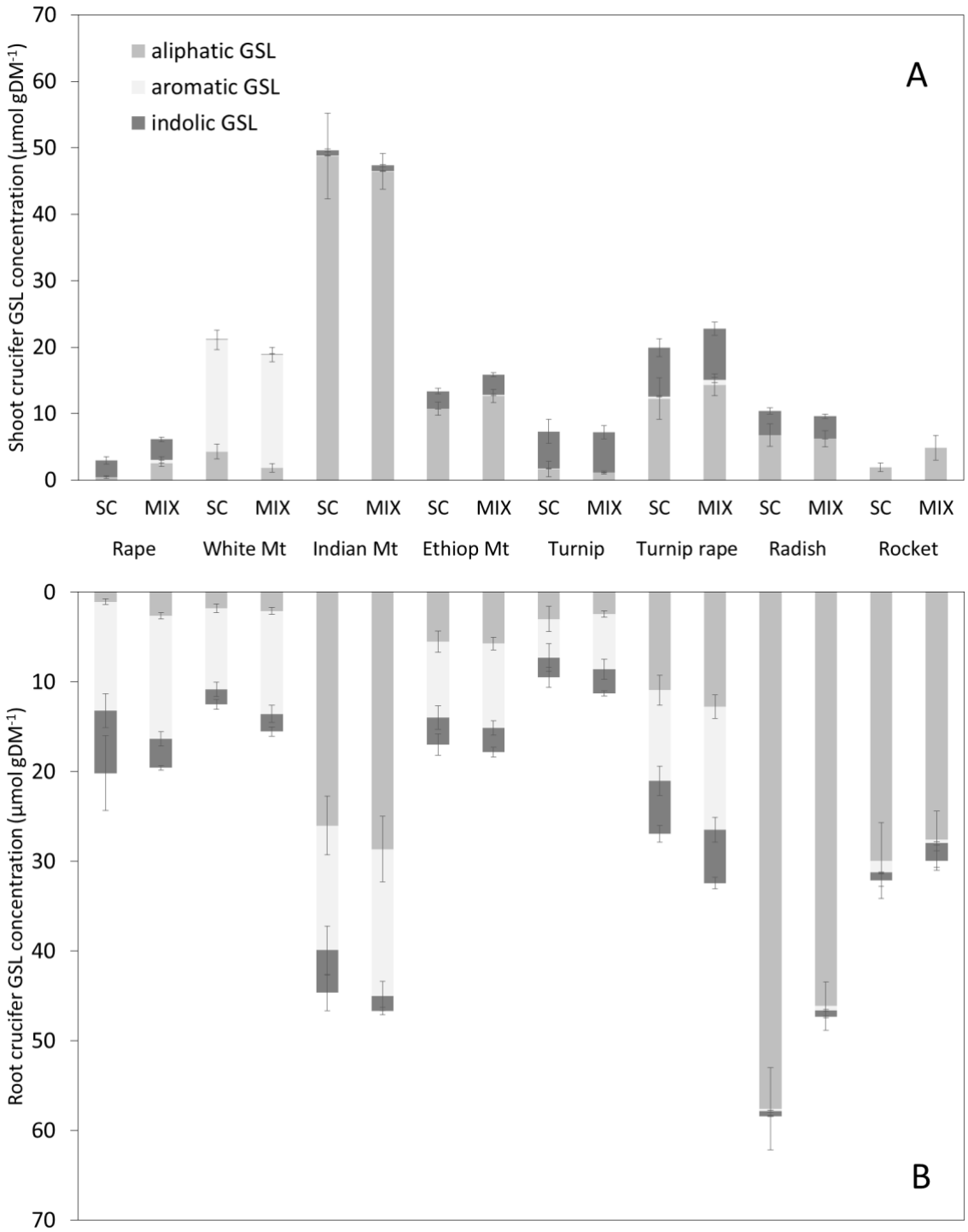

Figure 22. Mean root (A) and shoot (B) GSL concentration (µmol gDM^{-1}) of crucifer species sole cropped (SC) or in a mixture (MIX). Bars indicate the standard error.

Table 19. Individual glucosinolate concentrations (µmol gDM^{-1}) found in roots (R) of crucifer cover crops. Values correspond to the mean of the four experimental years for crops grown as sole crops and in mixtures. '0' means that GSL were under the threshold of detection. Numbers in brackets represent standard errors.

GSL name	Rape	White Mt	Indian Mt	Ethiopian Mt	Turnip	Turnip rape	Radish	Rocket
Root biomass (tDM ha^{-1})	0.54 (0.03)	0.38 (0.04)	0.36 (0.03)	0.37 (0.03)	0.67 (0.07)	0.63 (0.04)	0.84 (0.04)	0.07 (0.01)
Aliphatic								
Sinigrin	0.1 (0.03)	0.7 (0.14)	23.9 (2.15)	5.1 (0.51)	0	0.1 (0.04)	0.2 (0.07)	0.6 (0.24)
Glucoerucin	1 (0.09)	0.9 (0.17)	0.5 (0.19)	0.3 (0.12)	0.1 (0.07)	0.6 (0.07)	0.3 (0.04)	25.5 (2.39)
Glucoraphanin	0.2 (0.13)	0.1 (0.03)	0	0.1 (0.04)	0	1.7 (0.4)	0	1.8 (0.83)
Glucoraphenin	0	0	0	0	0	0	0	0
Glucoraphasatin	0	0	0	0	0	0	48.9 (2.56)	0
Gluconapin	0.1 (0.03)	0	0.1 (0.03)	0	0	1.8 (0.38)	0	0
Progoitrin	0.3 (0.06)	0.2 (0.08)	0.4 (0.29)	0.2 (0.09)	1 (0.25)	7.2 (0.85)	0	0.1 (0.08)
Glucobrassicanapin	0.5 (0.12)	0	0	0	0.3 (0.08)	1.3 (0.43)	0	0
Gluconapoleiferin	0.1 (0.02)	0	0	0	1.2 (0.11)	0.2 (0.06)	0	0
Glucoalyssin	0.1 (0.02)	0	0	0	0	0	0	0
aliphatic 6,37	0.4 (0.1)	2.1 (0.2)	0.1 (0.05)	0.3 (0.07)	0.1 (0.04)	0.3 (0.06)	0	4.5 (1.13)
Aromatic								
Sinalbin	0	4.8 (0.29)	0.1 (0.04)	0.1 (0.03)	0 (0.02)	0	0	0.1 (0.05)
Gluconasturtiin	13.6 (0.74)	5 (0.35)	15.4 (1.09)	9 (0.67)	5.7 (0.87)	13.8 (0.92)	0.4 (0.08)	0.5 (0.18)
Glucotropaeolin	0	1.5 (0.18)	0	0	0	0	0	0
Indole								
4hydroxyglucobrassicin	0.1 (0.02)	0	0.1 (0.02)	0.1 (0.02)	0.7 (0.12)	0.5 (0.1)	0.1 (0.01)	0
Glucobrassicin	0.5 (0.06)	0.1 (0.02)	0.3 (0.04)	0.8 (0.21)	0.4 (0.07)	0.8 (0.07)	0.3 (0.05)	0
4methoxyglucobrassicin	0.2 (0.01)	0.1 (0.02)	0 (0.01)	0.1 (0.02)	0.2 (0.06)	0.3 (0.06)	0.2 (0.02)	0
Neoglucobrassicin	2 (0.13)	0.3 (0.03)	0.9 (0.19)	1.4 (0.26)	1.1 (0.18)	3.3 (0.32)	0.1 (0.02)	0.1 (0.06)
Indole 16.3 unknown	1 (0.69)	1.1 (0.28)	1.2 (0.44)	0.3 (0.11)	0.1 (0.07)	0.2 (0.1)	0	1.6 (0.86)
indole 18,503	0	0	0	0	0	0	0	1.3 (0.5)
indole 15.683	0	0	0	0	0	0	0.9 (0.15)	0

Table 20. Individual glucosinolate concentrations (µmol gDM^{-1}) found in shoots (S) of crucifer cover crops. Values correspond to the mean of the four experimental years for crops grown as sole crops and in mixtures. '0' means that GSL were under the threshold of detection. Numbers in brackets represent standard errors.

GSL name	Rape	White Mt	Indian Mt	Ethiopian Mt	Turnip	Turnip rape	Radish	Rocket
Shoot Biomass (tDM ha^{-1})	1.45 (0.07)	1.47 (0.08)	1.00 (0.06)	1.43 (0.07)	0.7 (0.07)	1.31 (0.06)	1.45 (0.09)	0.68 (0.12)
Aliphatic								
Sinigrin	0.2 (0.23)	1.4 (0.25)	47.6 (1.89)	12.1 (0.82)	0	0	0.5 (0.14)	0
Glucoerucin	0	1.1 (0.25)	0	0	0	0	0	0.6 (0.17)
Glucoraphanin	0.3 (0.08)	0	0	0	0	1.1 (0.32)	0	2.4 (1.5)
Glucoraphenin	0	0	0	0	0	0	3 (0.51)	0
Glucoraphasatin	0	0	0	0	0	0	2.6 (0.26)	0
Gluconapin	0.6 (0.13)	0	0.3 (0.05)	0	0	8.8 (1.06)	0	1.2 (0.21)
Progoitrin	0.3 (0.15)	0	0	0	0.1 (0.06)	4.4 (0.54)	0	0
Glucobrassicanapin	0.5 (0.15)	0	0	0	0	1.5 (0.34)	0	0
Gluconapoleiferin	0.1 (0.02)	0	0	0	1.1 (0.24)	0.1 (0.04)	0	0
Glucoalyssin	0.2 (0.04)	0	0	0	0	0	0	0
aliphatic 6,37	0	2.3 (0.31)	0	0	0	0	0 (0.01)	9.3 (2.29)
Aromatic								
Sinalbin	0	12.6 (0.49)	0	0	0	0	0	0
Gluconasturtiin	0.5 (0.42)	0	0	0.1 (0.12)	0.1 (0.03)	0.6 (0.2)	0	0
Glucotropaeolin	0	3.9 (0.32)	0	0	0	0	0	0
Indole								
4hydroxyglucobrassicin	0.1 (0.01)	0	0	0.1 (0.01)	0.9 (0.29)	0.2 (0.05)	0	0
Glucobrassicin	1.5 (0.15)	0	0.1 (0.03)	2.1 (0.2)	1.4 (0.17)	1.8 (0.16)	3.3 (0.25)	0
4methoxyglucobrassicin	0.2 (0.01)	0	0	0.1 (0.01)	0.4 (0.04)	0.2 (0.03)	0.2 (0.01)	0
Neoglucobrassicin	1.3 (0.21)	0	0.6 (0.07)	0.7 (0.08)	3.2 (0.51)	5.5 (0.65)	0	0
Indole 16.3 unknow	0	0	0	0	0	0	0	0
indole 18.503	0	0	0	0	0	0	0	12.5 (1.72)
indole 15.683	0	0	0	0	0	0	0	0

3.2. Biomass production of cover crops

The mean LER_bT was 0.7 and was significantly greater than 0.5 in all experiments (0.66 in L2015 to 0.76 in V2014), demonstrating that crucifers had increased the biomass per plant in mixtures compared to sole cover crops ($P<0.001$). Overall, 14 of the 20 different mixtures had a LER_bT significantly greater than 0.5, while the remaining 6 mixtures had a LER_bT not different from 0.5 (Figure 23A).

The superior total per-plant crucifer biomass in mixtures derived from improvements in both the root (Figure 23C) and the shoot (Figure 23B) biomass responses. The mean LER_bR was 0.67 (range 0.6 to 0.8) and crucifers had LER_bR greater than 0.5 in 11 of the 20 mixtures, with none less than 0.5 (Figure 23C). Likewise the mean LER_bS was 0.73 (range 0.68 to 0.82) with 13 of the 20 mixtures significantly greater than 0.5, and none significantly less than 0.5 (Figure 23B). In summary crucifers had better root, shoot and total biomass per plant in mixtures than in sole cover crops.

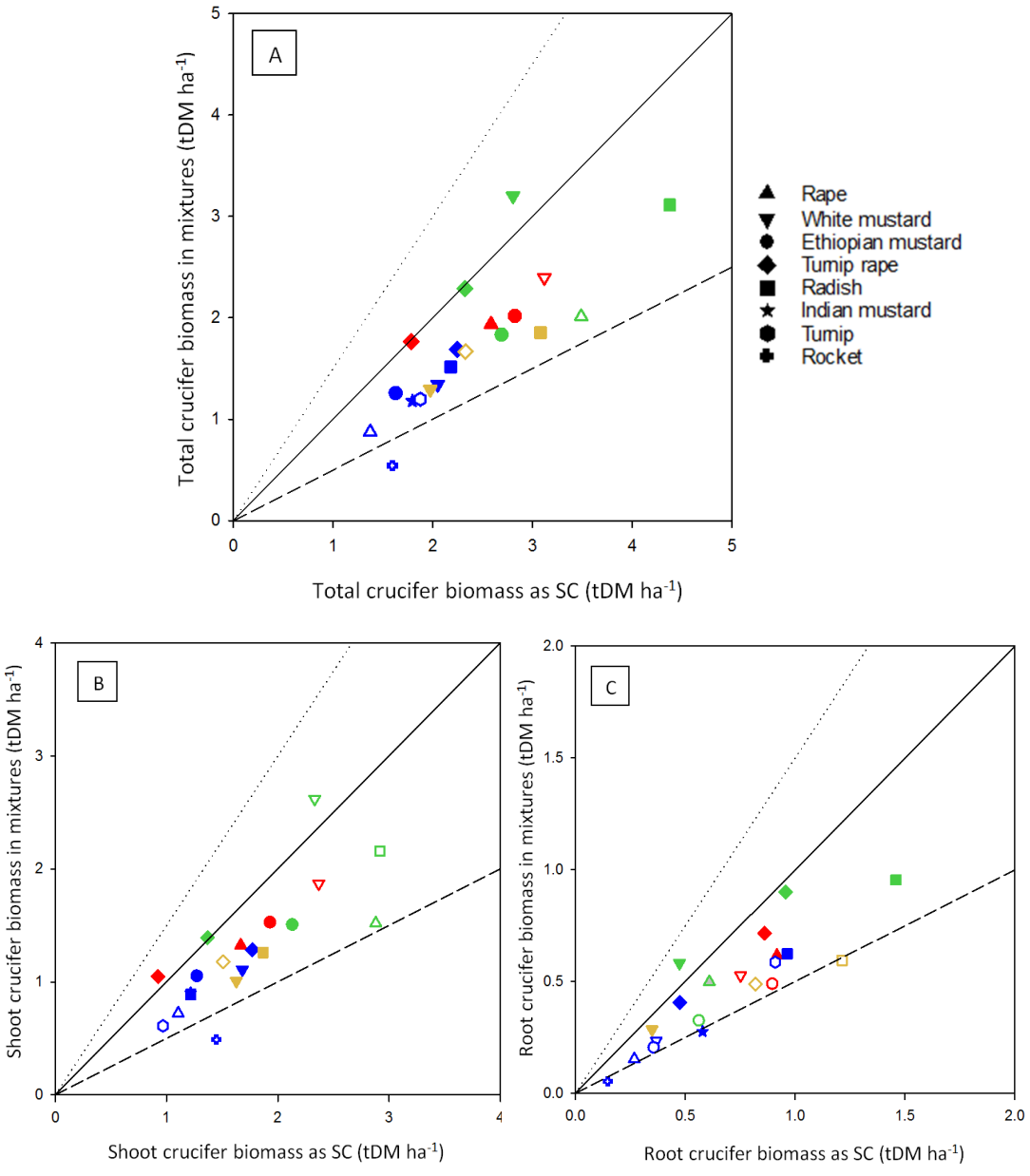

Figure 23. Crucifer biomass in mixtures (tDM ha^{-1}) (y-axis) as a function of crucifer biomass as sole crop (x-axis) for total plant (A), shoot (B) and root (C). The solid line represents LER_b =1 (y=x). Dotted lines represent y=1.5x and y=0.5x. Filled symbols and open symbols mean that the value is respectively significantly and not significantly different from the y=0.5x line (P<0.05). Colours vary by experimental site (V2014 in red, V2015 in yellow, L2014 in green, L2015 in blue).

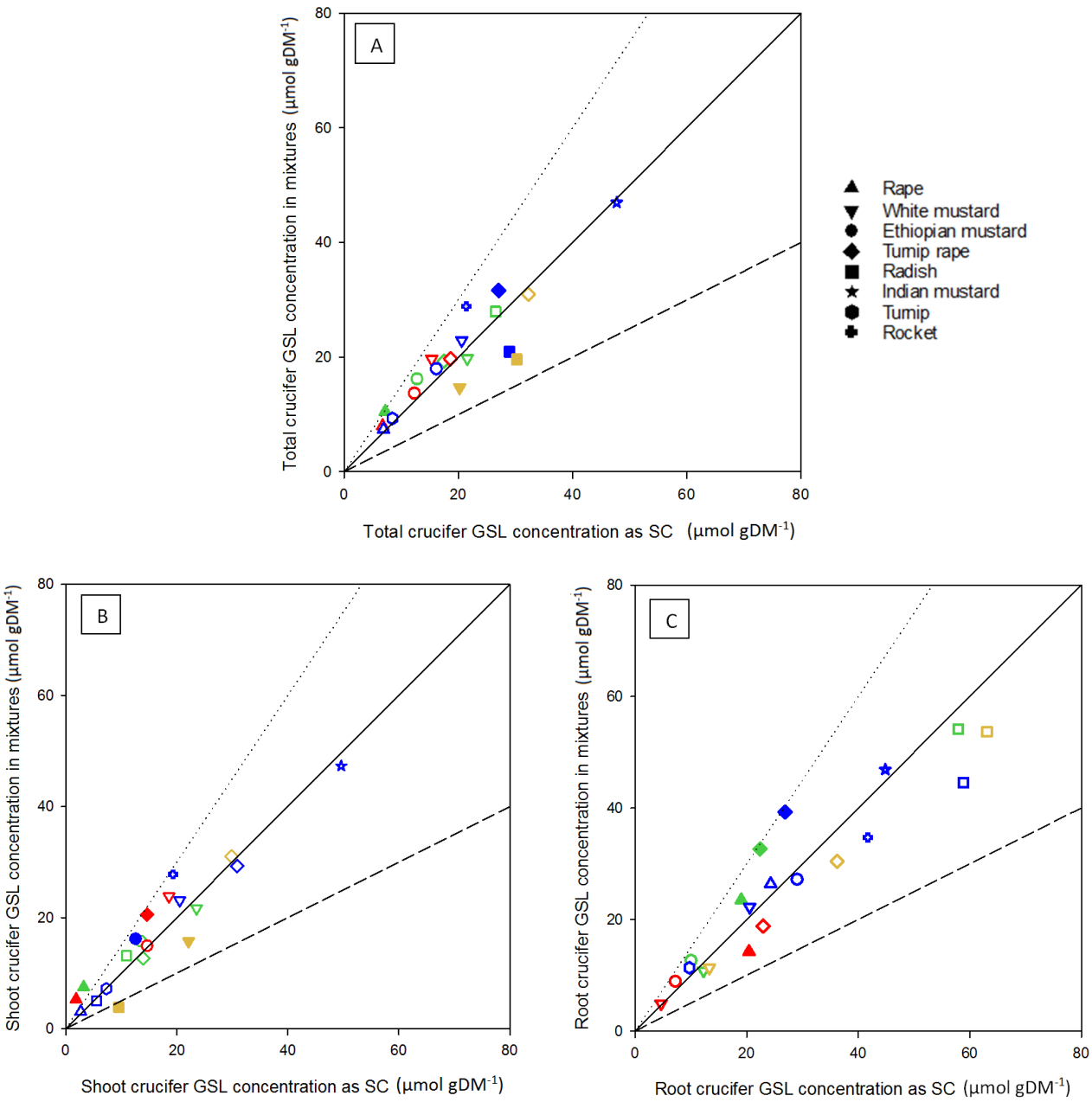

Figure 24. Crucifer GSL concentration (µmol gDM^{-1}) in mixtures (y-axis) as a function of crucifer concentration as sole crop (x-axis) for total plant (A), shoot (B) and root (C). The solid line represents $[GSL]_{MIX}$:$[GSL]_{SC}$ =1 (y=x). Dotted lines represent y=1.5x and y=0.5x. Filled symbols and open symbols mean that the value is respectively significantly and not significantly different from the y=x line (P<0.05). Colours vary by experimental site (V2014 in red, V2015 in yellow, L2014 in green, L2015 in blue).

3.3. Glucosinolate concentration of cover crops

The mean {$[GSL]_{MIX}$:$[GSL]_{SC}$} ratio was 1.1 and was not significantly greater than 1 (P=0.052) indicating that the concentration of GSL overall was only slightly modified and mostly not different between the mixtures and sole crop. The exceptions were rape (1.43) and turnip rape (1.25), in L2014, where the {$[GSL]_{MIX}$:$[GSL]_{SC}$} ratio significantly exceeded 1.0, and for radish in L2015 (0.76) and V2015 (0.65), white mustard in V2015 (0.73) where the ratio was significantly less than 1.0 (Figure 24A).

Concerning root GSL, the mean {$[GSL]_{MIX}$R:$[GSL]_{SC}$R} ratio (1.02) did not differ from 1.0 and only rape in L2014 (1.22) and turnip rape in L2014 (1.42) and L2015 (1.46) had a {$[GSL]_{MIX}$R:$[GSL]_{SC}$R} ratio significantly superior to 1.0 while rape (0.71) in V2014 had a ratio significantly less than 1.0 (Figure 24C).

In contrast to the root GSL, the mean {$[GSL]_{MIX}$S:$[GSL]_{SC}$S} ratio was 1.6 and was significantly greater than 1.0 (P<0.001), indicating that overall, shoot GSL was higher in the mixture than in the sole cover crop. However only rape in L2014 (2.47) and V2014 (2.72), turnip rape (1.50) in L2014 and Ethiopian mustard (1.29) in L2015 had {$[GSL]_{MIX}$S:$[GSL]_{SC}$S} ratios significantly greater than 1.0 while only white mustard (0.71) and radish (0.40) in V2015 had ratios significantly less than 1.0 (Figure 24B).

3.4. Glucosinolate production of cover crops

The mean LER_{GSL}T was 0.81 and was significantly greater than 0.5 (P<0.001) ranging from 0.54 in V2015 to 1.02 in L2014, indicating that crucifers had a significantly greater total GSL production per plant in mixtures than in sole cover crops. Overall 8 of the 20 mixtures had a LER_{GSL}T significantly greater than 0.5, while none of the mixtures had a LER_{GSL}T significantly less than 0.5. (Figure 25A).

The superior total per-plant crucifer GSL production in mixtures derived from improvements in both the root (Figure 25C) and the shoot (Figure 25B) GSL production responses. The mean LER_{GSL}R was 0.70 (range 0.54 in V2015 to 0.98 in L2014) and mean LER_{GSL}S was 0.90 (range from 0.55 in V2015 to 1.77 in V2014) and both were significantly greater than 0.5 (P<0.001). This demonstrates that crucifers had better root and shoot GSL production per plant in mixtures than in sole cover crops. The LER_bR was greater than 0.5 in 6 out of 20 mixtures (Figure 25C) and LER_bS in 7 out of 20 (Figure 25B) while no mixtures had ratios less than 0.5 for either root or shoot tissues.

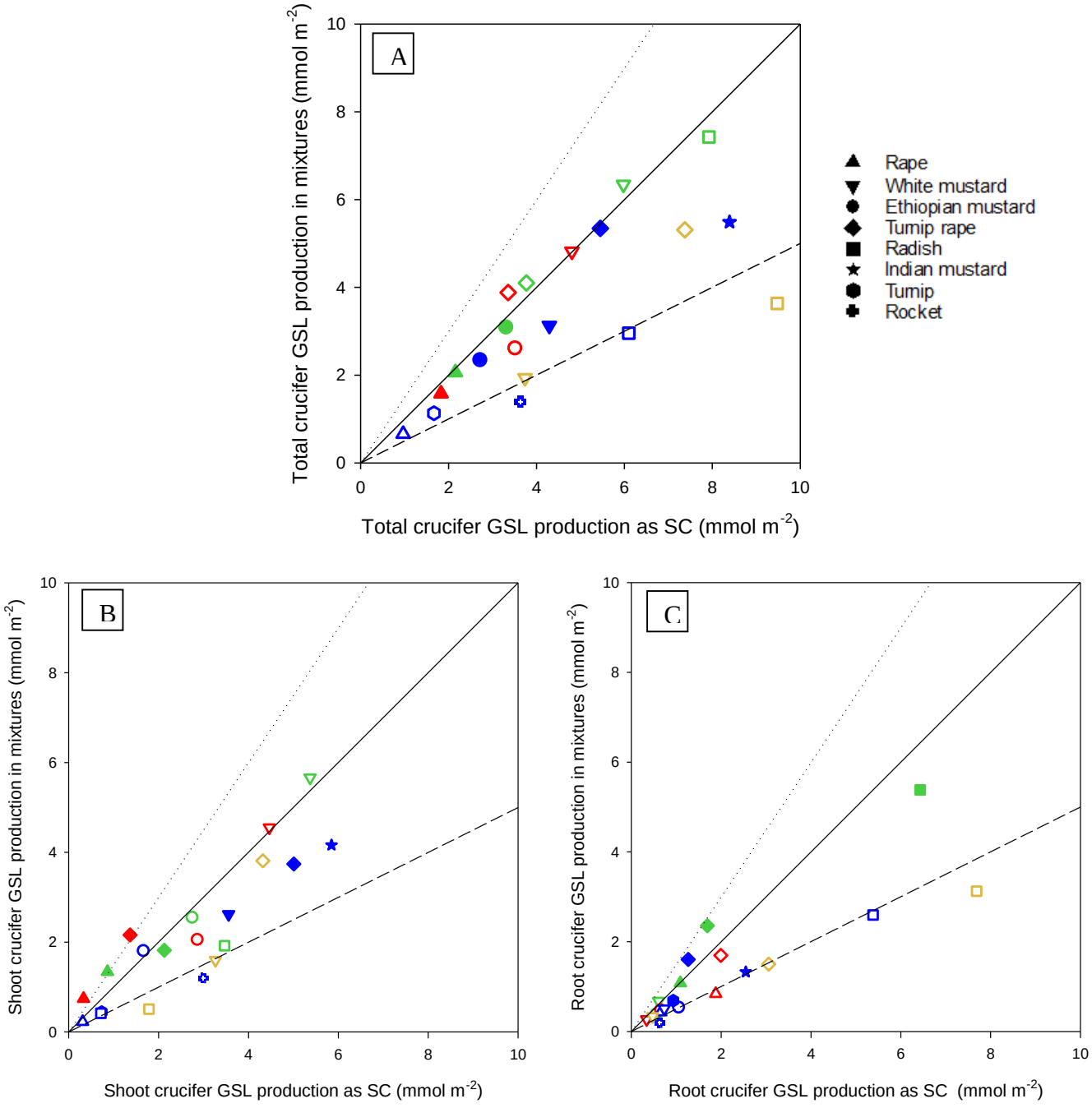

Figure 25. Crucifer GSL production (mmol m^{-2}) in mixtures (y-axis) as a function of crucifer production as sole crop (x-axis) for total plant (A), shoot (B) and root (C). The solid line represents LER_{GSL}=1 (y=x). Dotted lines represent y=1.5y and y=0.5x. Filled symbols and open symbols mean that the value is respectively significantly and not significantly different from the y=0.5x line (P<0.05). Colours vary by experimental site (V2014 in red, V2015 in yellow, L2014 in green, L2015 in blue).

4. Discussion

4.1. GSL profile and concentration

Our study aimed to assess the impacts on GSL types, concentration and production when crucifer cover crops were grown in bispecific mixtures with legumes compared with sole cover crops. Overall, crucifers in mixtures had the same GSL types, concentrations and proportions (aliphatic, aromatic and indole) as in sole cover crops. These results are in contrast to those of previous studies where the GSL concentrations in crucifer mixtures were monitored. Björkman et al., 2008 found decreases in aliphatic and indole GSL concentration when cabbage was intercropped with clover in an additive design, while Stavridou et al. (2012) found a decrease in indolyl GSL in broccoli florets when broccoli was intercropped with lettuce in a substitutive design. Plant-plant interactions occurring in mixtures change N and S availability for the crucifer species and it is well known that GSL composition is highly dependent on crop S and N concentration (Omirou et al., 2011; Zhao et al., 1994). Several studies on sole/pure crops have shown that a better S supply, by fertilisation, increased the aliphatic:indolic GSL ratio (Falk et al., 2007) while increased N supply increased the indolyl GSL concentration (Stavridou et al., 2012a). We found similar aliphatic:indolic ratio in our experiments for a wide range of crucifer-legume species which suggests that mixtures have not affected the crucifer N:S ratio. Therefore under our experimental conditions crucifer species could have competed with the legumes to the same extent for both N and S pools. Indeed on the same experiments, Couedel et al. (2018) found that crucifer competed very effectively with the legumes for both N and S supplies. Better biomass per crucifer plant was recorded in our experiment and may have led to a dilution effect of N and S (Colnenne et al., 1998; Mathot et al., 2009) but GSL concentration was not affected. Given there was little impact of either crucifer variety or legume species on GSL profile and concentration, the effect appears to be quite consistent and general across plant types and environments with only rare examples of departure from the general responses. GSL profiles are conserved among growing conditions, while concentrations can be influenced by a range of factors – but here the environment of the bispecific mixture has not generated great changes in GSL concentration. We may hypothesise that N and S nutrition status of crucifers in mixtures were not affected and that threshold concentrations of secondary metabolites such as GSLs are conserved as they are necessary to maintain a given protective or attractant function, and may have strong evolutionary attractant or suppressive roles in the plant (Van Dam et al., 2009).

4.2. GSL production

Our results show that crucifers in mixtures tend to have a better biomass per plant, both for shoots and roots, than in sole crops for a wide range of species and growing conditions. This increased biomass demonstrates the capacity of crucifers to compete strongly with legumes for abiotic resource acquisitions, which has been reported by numerous previous studies both in cash crops (Andersen et al., 2005; Szumigalski and Van Acker, 2008) and cover crops (Tribouillois et al., 2016a; S. E. Wortman et al., 2012). We have demonstrated in this study that despite the higher crucifer biomass per plant, the GSL types and concentration remained relatively unchanged, with no GSL 'dilution effect' per plant. As a result, the total GSL production per plant was generally increased in the mixture compared to the sole cover crop. In the substitutive bispecific designs, the density of crucifers was halved compared to sole crop, yet the GSL production on an area basis only declined by 19% for the whole crucifer plant and 30% less in the roots but only 10% less in the shoots. In this regard, the potential of crucifer GSL production from the roots during growth, and the biofumigation potential of the final crucifer biomass may have been maintained at higher levels than would be predicted by simple 50% dilution of the sole crop, so that pest-suppressive eco-system services may have been maintained at higher than predicted levels. Even if biofumigation represents a promising way to control pests in cropping systems, we report a lack of knowledge on the pest suppression effects of crucifer-legume mixtures. Specific experiments are thus needed in order to clarify these potential biofumigation effects of crucifers, cultivated in sole cover crop or in mixtures, on a wide range of pests.

4.3. Ecological and agronomic implications

Suppression of cover crop pests and diseases are key to maintaining cover crop biomass, and thereby to maintain other services such as nutrients cycling, soil and water quality. In an ecological sense, compared to sole/pure crops, the unchanged GSL profile and concentrations produced by crucifers in mixtures mean that normal levels of pest and disease suppression (or attraction) can be expected during the growing period, with no greater susceptibility linked to lower levels of GSL concentrations in either root or shoot tissues. Furthermore it has been shown in some studies that during the growing period, crucifer-legume mixtures provide better defence against crucifer pests and insects than pure crucifer sole crop (Reddy, 2017) due to a lower crucifer density and by both physical and chemical disturbance (Caamal-Maldonado et al., 2001; Ratnadass et al., 2012) induced by the legume. In the same way,

crucifers in the mixture can also reduce legume diseases (Boudreau, 2013) which in turn ensure a high level of N related ecosystem services provided by the legume. Overall in case of diseases and pests outbreaks, mixtures may be potentially more resilient than either crucifers and legume sole crops to reduce the likelihood that the cover crops themselves are attacked by pests and diseases. T hereby mixtures may maximise the other eco-services that are linked to adequate biomass production of the legume (N- and S-related services) and the crucifer (biofumigant services, without disservices).

Suppressions of weeds, pests and diseases of subsequent cash crops can rely on GSL production on a ground area basis (Matthiessen and Kirkegaard, 2006) in order to achieve a threshold soil concentration that is effective for specific pest species. In this regard, crucifer GSL production per area basis were maintained above the levels anticipated by the 50:50 substitutive mixtures with only a 20% reduction in GSL production and hence biofumigation potential, assuming no negative interaction occurs with legume residues. Increasingly, pests suppressive effects even of sole/pure crucifer stands have been linked to non-GSL related mechanisms that relate to more general changes to the soil biological community (Kirkegaard, 2009; N. Motisi et al., 2009b; Omirou et al., 2011; Vukicevich et al., 2016). Beyond GSL related effects, mixtures have also been show to better suppress pathogens than sole/pure crops following cover crop termination and incorporation (Boudreau, 2013; Reddy, 2017). Indeed in mixtures, legumes can generate a more favourable environment for beneficial fungi and microbial diversity thanks to a higher nutrient supply than crucifer sole crops (Vukicevich et al., 2016). Other specific chemicals released by legumes (including H_2 gas and phenolic acids) can also have suppressive effects on pests and diseases (Mondal et al., 2015; Wink, 2013b) or act indirectly by encouraging the populations of other suppressive organisms. Non legume – legume mixtures have been shown to better suppress weeds by strong competition for light and nutrient acquisition compared to sole crop (Altieri et al., 2011; Corre-Hellou et al., 2011). Further studies are required to assess cover crop mixtures for potential to suppress pests and diseases, but our study demonstrates that GSL production has been maintained at higher than predicted levels based on the plant density in the cover crop mixtures.

In a crucifer legume cover crop mixture, levels of biofumigation and N recycling services are mainly driven by crucifer and legume biomass production respectively. Limitations of plant-plant competitions are then key to reach high levels and optimise multiple ecosystem services. Several studies have indicated that the best complementarity for mustard-legume

mixtures occurred when the mustard was sown at a density less than 50% of its sole crop density, and when the legume was sown at a density more than 50% of its sole crop density (Kushwaha, 1987; Wendling et al., 2017). Indeed both interspecific competitions and potential allelopathic effects on legume could decrease with lower crucifer density. In this regard it would be relevant to test the effects of different cover crop component species density to assess their impact on both crucifer GSL production and legume biomass in order to find the best compromise between services of biofumigation and N and S cycling.

In order to broaden the spectrum of impact on pests and diseases, more than one crucifer could be used in mixtures to produce a higher diversity of GSL type. In crucifer-crucifer mixtures, GSL production of each species are unknown, but in terms of biomass production, Wortman et al. (2012) highlighted a high competition of mustard on other crucifer species. Indeed competition between crucifer species for N and S resources could be high and asymmetric and as a result, strongly influence the GSL production of both species. Studies are then needed to assess GSL production in crucifer species mixtures if the suppression of a high diversity of pests and diseases is targeted. Nevertheless crucifer-crucifer mixtures could also create lower production of a specific biocidal GSL (e.g. Sinigrin) and we can hypothesise a decrease of biocidal effects of the whole cover on a specific pathogen. Indeed some pathogens are specialists and highly sensitive to the type of GSL hydrolysis products (Matthiessen and Kirkegaard 2006). Nevertheless in field conditions there appears to be a lack of knowledge on threshold of GSL production required to achieve suppression of a specific pathogen as it depends of many variables such as soil type, climate and pathogen cycle (Motisi et al., 2010).

5. Conclusion

Our study is the first to our knowledge to report the plant-plant interaction effects on GSL profiles in bispecific crucifer-legume cover crop mixtures. As a result of a better biomass production per plant without changes in GSL types and concentration, total GSL production per crucifer plant was significantly increased in mixtures. On a ground area basis, despite half the density of crucifers, the cover crop mixtures produced only 20% less GSL compared to sole crucifer cover crops. Unchanged GSL type and concentration means that crucifers have the same interactions with pests and diseases during the growing period. These results have been validated for a wide range of crucifer species and support the use of crucifers and legumes in mixtures to achieve multiple ecosystem services. Even if the GSL production on

an area basis declined by only 20% and the GSL profile maintained, specific experiments are needed to evaluate the impact of this dual effect on biofumigation potential of mixtures compared to sole crucifer cover crops.

Chapter 6. General discussion, conclusion and research perspectives

The overall objective of the thesis was to simultaneously study services provided by cover crops linked to N, S cycle, GSL production and biocontrol. The originality of our work was to assess both biotic and abiotic services while studies usually assess one service at a time. In particular we bring significant improvement and cast light on S cycle in mixed cover cropping (Chapter 4) and highlight an area of research that need to be deeply investigated, such as the impact of legume inclusion in crucifer stands to suppress pathogens (Chapter 2).

In this general discussion chapter we firstly gather main results from the overall thesis and propose agronomic innovations to increase the expression of multiservice by their mutualisation. In a second part we study interactions between N, S and GSL production for crucifers in sole crop and in mixtures. In a third part we consider research approaches to fill the considerable knowledge gaps in biofumigation and biocontrol topics. We also propose strategies to improve cover crop choice, breeding and research efforts to support the implementation of the multiservice approach.

1. Mixtures as a way to increase services and avoid disservices compared to sole cover crops.

1.1. Cover crops mixtures mutualize N, S cycle's services and GSL production compared to sole cover crops.

Results described in chapters 3 and 4 showed that mixing crucifer and legume cover crops species allowed a mutualisation of N and S cycle services with a high level of expression (Figure 26). Species mixtures depleted in the same extent soil N and S (catch crop services) while significantly increased the N recycling when mixtures are terminated (green-manure service) compared to that of crucifer sole cover crops. Interestingly, crucifer GSL production per area basis were almost maintained above the levels anticipated by the 50:50 substitutive mixtures, with only a 19 % reduction in GSL production (Figure 26).

Despite half of crop density for crucifers in mixtures their biomass reached in average 70% per basis area compared to their crucifer sole cover crops (Chapter 5). Therefore biomass production increased per crucifer plants but no dilution phenomenon occurred either for N, S and GSL production as it could have been be expected when biomass increases (Colnenne et

al., 1998; Mathot et al., 2009). These high performances of crucifer in mixtures due to complentarity for the capture of resource and facilitation are the main explanation of high N and S catch crop services provided by the species mixture of crucifer and legume.

Besides services, cover crop mixtures seems above all interesting to mitigate disservices. Indeed all species mixtures tested did not allow to increase services compared to the best family of cover crops for given service but rather decrease services of poor N green manure by crucifer sole crops and poor S green manure by legume sole crops (Figure 26). Bundle of services approaches have been highlighted by Finney et al. (2017) who concluded that "functionally diverse mixtures increased multifunctionality by ameliorating disservices associated with component species, not by enhancing services".

	Crucifer sole crops	Legume sole crops	Crucifer + legume
N catch crop	100%	66%	98%*
N green manure	18%	100%	63%*
S catch crop	100%	30%	99%*
S green manure	100%	23%	85%*
GSL/ha	100%	/	81%*

Figure 26. Scheme summarizing levels of services provided by cover crop mixtures compared to sole cover crops (see details in chapter 3, 4 and 5). Services are provided in proportion of the best sole crop family (average of all species tested), which then is considered at 100%. * means that mixtures have a level of service significantly higher than the average of sole cover crops.

Another interesting point often cited as a key feature of species mixtures is the gain in yield or services in stability compared to pure crops (Raseduzzaman and Jensen, 2017). Indeed in mixture a failure of one species is less impactful on the overall biomass and then on services than in pure crops, illustrating that species mixtures, in particular for cover crops, give a security for the success of emergence, growth and capture of nutrients. Very few studies deal

with this stability perceptive in the literature, Smith et al. (2014) and Wortman et al. (2012) found that mixtures tend to reduce biomass variably over sites and years but results were rarely significant. In our study when pooling all data from our 4 site × year experiments, we did not find a lower variability of services performances in species mixtures compared to the best pure crops, expected for the N green manure service compared to pure crucifers.

In conclusion and in our conditions, the mutualisation of services is the key asset of cover crop mixtures, and much more than service stability, over sites and weather inter-annual variability.

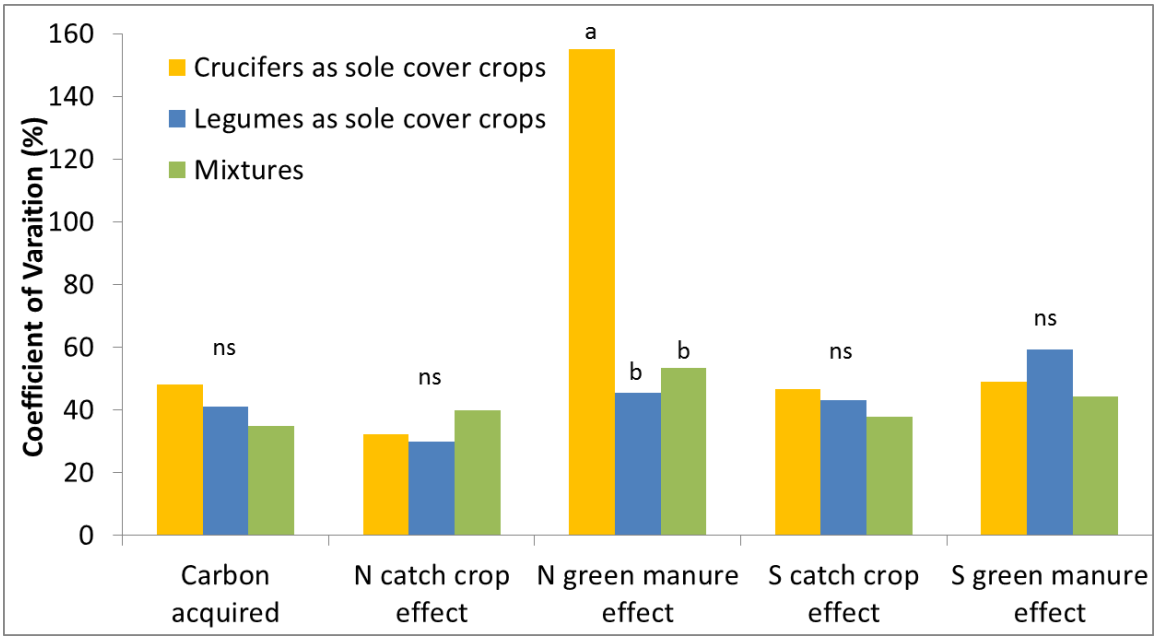

Figure 27. Coefficient of variation (%CV) of ecosystem services provided by cover crops on our 4 sites × years. Kruskall-Wallis non parametric tests were used to test differences between treatments. Different letters above bars indicate a significant difference in CV values ($P<0.05$).

1.2. Which type of competitions or complementarity between species?

In our work we used the land equivalent ratio (LER) method (Bedoussac and Justes, 2011) to assess performances of cover crop mixture versus sole crops, and analyse the level of complementarity of between species in mixtures. This method is often used for yield and biomass production of cash crops (Andersen et al., 2007; Bedoussac and Justes, 2010) and cover crops (Wendling et al., 2017) but has been scarcely used for nutrient acquisition excepted few studies (Tribouillois et al., 2016a). We used LER approaches in mixtures for N uptake, S uptake and above all for a secondary metabolite (GSL) for first time at our

knowledge. Complementarity and competition underlying species mixtures performances were assessed in chapter 3, 4 and 5. We found that for both N and S services, no incompatibility was observed in the mixtures tested, despite the high and variable competition for abiotic resources generated by crucifers in the cover crops. Crucifers clearly dominated mixtures for biomass, N and S uptake while legumes were either not advantaged or slightly penalized. Indeed we reported that legumes were disadvantaged only when mixed with radish and turnip (less N and S acquired per plant). Nevertheless, our experimental design did not enable us to separate effects from competitions for resources (nutrients, light and water) and effects from potential allelopathy (from ITC or other molecules). Strong tap root from both radish and turnip rape could explain such competitions via high capacity to uptake nutrient in soil and high root GSL production per basis area. Nevertheless, in the literature we saw that it exists a gap of knowledge on methods to understand causes of competitions and eventual allelopathy effect in crucifer legume mixtures. We discuss in section 2.1 of this chapter, some potential solutions to isolate GSL effects of crucifer on living organisms that can also be applied to study and measure the effects of GSL on companion legume. Competitions for other nutrients than N and S could also be another factor explaining levels of compatibility or incompatibility between species. Nevertheless, very few data exist in the literature on other nutrient uptake by both legume and crucifer sole cover crops, let alone in mixtures.

1.3. Unequal approaches for biocontrol issues

In Chapter 2 we study and update in the most exhaustive way possible biocontrol effects of crucifer pure crops on a wide range of pathogens through an extensive literature research. We highlighted 1) an inequality of knowledge depending on pathogens targeted not linked to the importance of the pathogen 2) a strong lack of consideration of the impact biocide molecule could have on essential beneficials. Furthermore, the interest of legume inclusion to increase biocontrol was mainly theoretically conceptualised as we report a strong gap of knowledge on the impact of crucifer legume crops for biocontrol purposes. Research effort should particularly be carried out on impact of mixtures on pathogenic fungi and bacteria where there is a dearth of data to understand mechanisms of suppression compared to sole cover crops. Impact of both mixtures and sole cover crops on slug populations is maybe the most important area to investigate as it often represent a strong obstacle for farmer to grow cover crops. Data on aerial beneficial macrofauna is also lacking both in sole cover crops and

mixtures while it could represent a strong incentive at the plot and landscape level to foster farmers to grow cover crops.

1.4. Toward innovation to increase and manage levels of services

Our work was only carried out with bispecific mixtures while more than 2 species are often used by farmers for cover cropping. Indeed, seed companies already sell mixtures including up to 12 different species to target various ecosystem services. The ecological assumptions of species complementarity for natural ecosystems are challenged in cover cropping experiments were more diversity doesn't necessary mean more quantity and quality of services (Denise M. Finney et al., 2017; Smith et al., 2014). Finney et al. (2017) and Finney and Kaye (2017) concluded that species number is less important than diversity of traits present in the mixture when targeting multifunctionality.

Applying this trait diversity approach to biofumigation properties, more than one crucifer could be used in mixtures to broaden the spectrum of impact on pathogens through higher diversity of GSL. GSL production still need to be studied in crucifer-crucifer mixtures as key GSL could see their production decreased due to high competitions for N and S and then engendering a decrease of biocidal effects of the whole cover (Figure 28). Therefore more crucifer diversity doesn't mean necessary more pathogens suppressed.

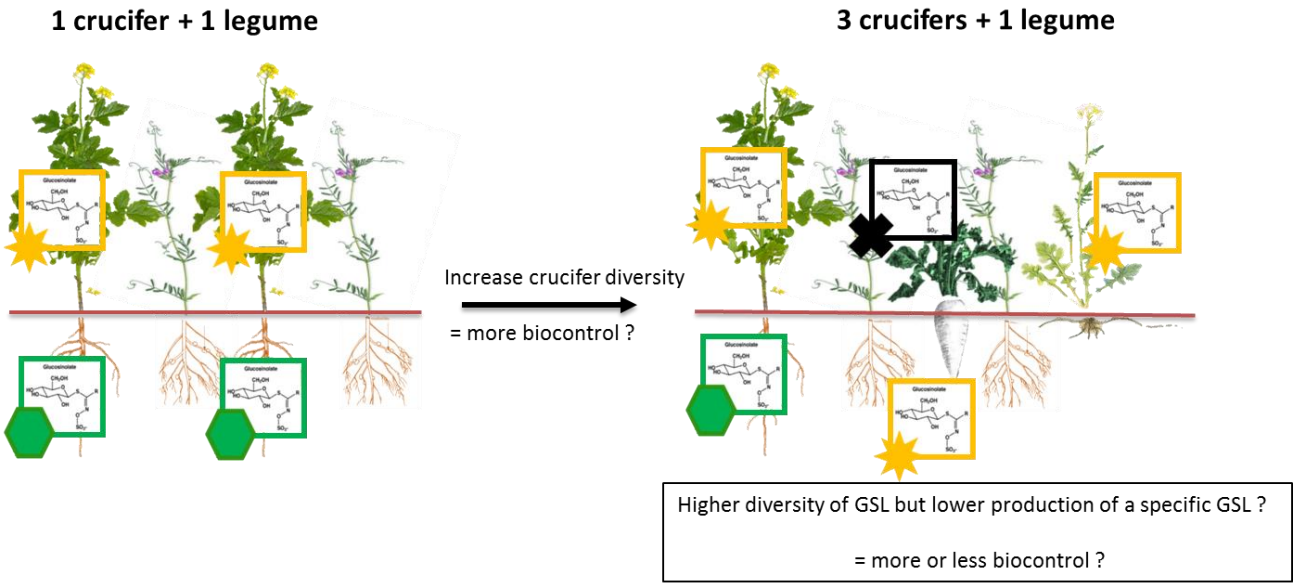

Figure 28. Increasing cover crop diversity doesn't necessary mean more services. Example on GSL biofumigation properties of crucifers.

Another way to maximize services through an agro-ecological approach is to have relay of services in time during the fallow period. For example Tribouillois (2014) showed that mixing a frost sensitive non legume with a non-frost sensitive legume allow to have a strong catch crop service before frost and then in relay a N high green manure effect as once the Moha frost-killed, the legume growth is boosted during autumn and winter. For crucifer-legume mixtures, a white mustard frost-killed around -7°C could be used with a crimson clover which is less sensitive to moderate frost in order to provide services in relay. Selecting crucifer cultivars more sensitive to frost could then be an interesting way to be able to design mixtures maximizing both N catch crop and N green manure services at the same level than the best of pure crop families, without using any herbicide to kill the cover crop.

Many agro-ecological options through species mixtures could then be created but the level of species complexity and choices in mixtures should always be thought according to services targeted (see this approach discussed in section 3.3). It is clear that research is needed to determine and model how to assemble species to have mixtures providing the multi-service targeted.

2. Modelling N, S and GSL

2.1. Synthesis of the literature

Even if mono-service studies are most of the time useful to identify and understand agronomic and biological mechanisms, studying links and trade-off between services is the next step to follow to optimize agro ecological performances (Denise M. Finney et al., 2017; Schipanski et al., 2014). N, S cycles and GSL production services are thus interesting to study conjointly as crucifer N and S uptake are linked and GSL production may depend on N and S plant status (Omirou et al., 2009; Rosen et al., 2005). GSL analysis are costly and not handy to carry out, therefore identifying links between their production levels through N and/or S analysis may save time and money. It has been shown that N and S supply can influence both crucifer N, S uptake but also GSL production (Li et al., 2007a; Schonhof et al., 2007). Some studies found negative correlation between N: S ratio and GSL concentration for broccoli (Schonhof et al., 2007; Stavridou et al., 2012a) and turnip crops (Li et al., 2007a). Explanations of this GSL concentration decrease when N: S ratio increases are that vegetative growth overtake GSL biosynthesis, diluting the content of these metabolites (Kim et al., 2002; Rosen et al., 2005). Interestingly Gerendás et al. (2009) reported that under excessive or severely limiting S status, sinigrin concentration in Indian mustard seeds does not depends

on the N: S ratio. Stavridou et al. (2012a) found significant positive correlation between S% and aliphatic or indolic GSL and Schonhof et al. (2007) found positive correlation between S% and glucoraphanin concentration in broccoli crops. As secondary metabolites, GSL production is also sensitive to critical plant stages, plant stresses or pathogen attacks (Björkman et al., 2011; Sarwar et al., 1998), explaining the difficulty to predict GSL production using simple relationships with N or S concentrations.

These is a strong need for studies dealing with cover cropping to analyse links between N and S status to better access to services of S according to N services in a bundle of services perceptive. Indeed, S capture is expected to be more closely linked to N capture since S and N are metabolized to produce some proteins but data are lacking to explain it for crucifers in cover cropping. Moreover, it would be interesting to explain GSL production via N, S status, and to taking into account phenological stages, as well as biotic and abiotic stresses. Finally, the analysis of the impact of growing crucifers in mixtures on plant relations between N, S and GSL contents are also key to study, as they may be different than sole crops due to interactions between plants to capture abiotic resources. Nevertheless, the only study we found on this topic was the one of Stavridou et al. (2012) who found no significant differences of N, S status and GSL production by comparing sole and mixed broccoli crops.

As N uptake, S uptake and GSL production are driven by multi-factors it could be relevant to analyse such relationships using generalized linear model (GLM) approach to understand the key variables and interactions governing the GSL production. Conceptual model for GSL production could then include explanatory variables such as biomass, N and S concentration, thermal time, development stages, nitrogen and water stresses.

Thanks to our 4 sites × year experiment data (see the Material and Methods section in Chapter 5) allowing to have a first dataset able to test this idea, we aimed to 1) analyse links between N and S status, and 2) explain GSL production via N: S ratio, phenological stages and nitrogen abiotic stresses.

As for every simple relationship between N, S and GSL no differences between crucifer plant in sole crops and mixtures were highlighted through t.test comparisons, in agreement with the only study we found on this topic from Stavridou et al. (2012), we gather data from sole crops and mixtures to do this analysis.

2.2. Links between N, S status and GSL content

The direct correlation between plant N% and S% was weak for the whole plant (R^2 = 0.22) and higher for roots (R^2 = 0.44) than shoots (R^2 = 0.17) (Figure 29). Relations are not clearer among species where no differences are observed (data not shown). These results are surprising as S capture could have been expected to be more closely linked to N capture since S and N are metabolized to produce protein but data are lacking to explain it for crucifer in cover cropping.

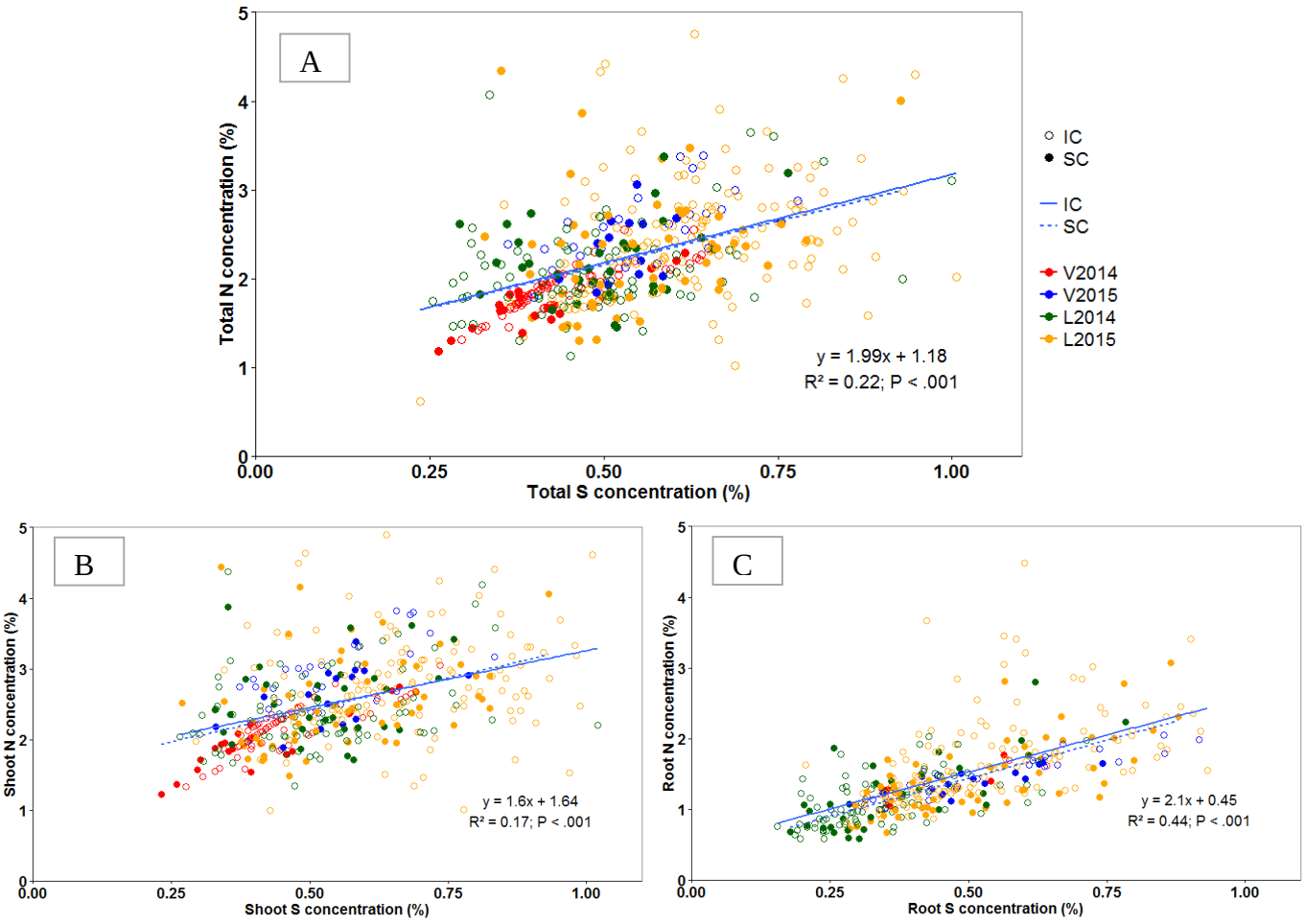

Figure 29. N concentration (%) of crucifers as a function of S concentration (%) for (A) the whole plant, (B) shoot parts and (C) root parts.

No correlation was found between GSL concentration and N: S ratio for the whole plant, shoots and roots. This result differs from other studies that found negative correlation between N: S and GSL concentration for broccoli (Schonhof et al., 2007; Stavridou et al., 2012a) and turnip crops (Li et al., 2007a). Explanations of this GSL concentration decrease when N: S ratio increases are that vegetative growth overtake GSL biosynthesis, diluting the content of these metabolites (Kim et al., 2002; Rosen et al., 2005). Interestingly Gerendás et al. (2009) reported that under excessive or severely limiting S status sinigrin concentration in Indian mustard seeds does not relate to the N: S ratio.

S% was poorly positively correlated with GSL concentration for the whole plant ($R^2 = 0.12$) and shoots ($R^2 = 0.12$), the correlation was even higher for roots ($R^2 = 0.34$) (Figure 6). Our results are then agree with Stavridou et al. (2012a) who found significant positive correlation between S% and aliphatic or indolic GSL and with Schonhof et al. (2007) who found positive correlation between S% and glucoraphanin concentration in broccoli crops.

2.1. Generalised linear model approaches to model S% and GSL production

2.3.1. Generalised linear modelling method

We used generalized linear modeling (GLM) to model S and GSL concentration. The stepwise regression procedure (Step AIC) allowed us to select variables (see section 2.2.2) to include in the final model using Akaike's Information Criterion (AIC) as a variable selection criterion. Backward selection was used in the stepwise procedure, which begins with all candidate variables. Resulting variables that correlated at $P > 0.05$ were rejected. GLM were fit using the statistical software package R (R Core Team, 2016). We used the "Modern Applied Statistics with S" (MASS) package to perform GLM and stepAIC functions.

In the conceptual model we hypothesis that S concentration would depend mainly on biomass, N concentration, thermal time and development stages. The initial conceptual model would be:

$S\ concentration_i = Biomass_i + N\ concentration_i + interaction\ [Biomass_i \times N\ concentration_i] + Thermal\ time + Development\ stages.$

With i correspond either for variables corresponding to Total, roots or shoots parts.

In the conceptual model to explain GSL concentration we choose variables such as biomass, S concentration, N concentration, N: S ratio, thermal time and development stages. As GSL concentration depends on abiotic stress, it may also depends on nitrogen nutrition indices (NNI). For NNI we choose the critical dilution curve of mustard (Dorsainvil, 2002) as a proxy for NNI of all crucifer species. We performed the initial model receptively for the whole plant, shots and roots as followed:

$GSL\ concentration_i$ = $Total\ biomass_i$ + $S\ concentration_i$ + $N\ concentration_i$ + $N{:}S\ ratio_i$ + interaction [$Total\ S\ concentration_i \times Total\ N\ concentration_i$] + Thermal time + Development stages + NNI

With i correspond either for variables corresponding to Total, roots or shoots parts.

2.3.2. Factors influencing S and GSL status

GLM analysis gave a weak model for explaining the relationship between main factors suspected to influence S% of the whole plant (R^2 = 0.21), or in shoots (R^2 = 0.28) but a stronger relationship was found for roots (R^2 = 0.57) with GLM including 3 variables (root N%; root biomass and time expressed in thermal-time).

Unfortunately this first GLM analysis of our dataset did not led to model GSL content using the main factors suspected their production and its variability observed neither for data of the whole plant (R^2 = 0.13), nor shoots (R^2 = 0.19). A significant GLM model, including 3 variables (root S%, root biomass and the interaction between them), was found significant for root GSL production (R^2 = 0.37) but it was not sufficiently correct for explaining the variability observed.

In conclusion, this first modelling study showed that S uptake and GSL production are driven by multifactor forces and are then difficult to predict using simple variables and statistical modelling approach. This indicates that a deeper analysis and physiological studies are needed to go further in the prediction of GSL production in cover cropping conditions.

3. Towards improved understanding of mechanisms behind pathogen suppression

This section corresponds to the third and last part of a review manuscript submitted in Advances in Agronomy as:

Couëdel, A. Alletto, A. Kirkegaard, J. Justes, E. Crucifer – legume cover crop mixtures for biocontrol: a new multi-service paradigm.

This review highlights the numerous mechanisms involved in pest suppression by cover crops and the importance of careful selection of appropriate cover crop species for use in mixtures. Several aspects of experimental design and approach must be considered to generate improved understanding of such mechanisms.

3.1. Isolating GSL effects

When using crucifers, it is important to know whether GSL production has an effect on the organisms of interest or not (e.g. Vervoort et al., 2014), as the choice of crucifer (or not), and an appropriate species and cultivar depends upon it. The use of non-GSL containing plants as controls, or more ideally crucifer cultivars with high and low concentrations of specific GSLs, are useful tools in this regard (Kirkegaard 2009). Many studies used low and high GSL-containing plants to determine GSL biocidal effects, but a control with a crucifer containing no GSL is ideal as the threshold of toxicity is unknown, and low GSL production could still engender biocidal effects. For example, canola double 0 (“00”) or canola quality Indian mustard cultivars producing very low seed GSL levels are often not suitable as GSLs are still produced in the shoot and root tissues in appreciable quantities (Kirkegaard et al., 2000), though some lines with close to undetectable levels of specific GSLs have been identified. Another approach used to create GSL-free crucifer effects is to transgenically remove the myrosinase cells, as shown for rape seeds by Borgen et al. (2010) and so limit the hydrolysis to ITCs. However myrosinase enzymes are also present in the soil and can transform GSL released from crucifers into biocidal compounds which can limit this approach in non-sterile soils (Gimsing and Kirkegaard, 2009). On the other hand, the biosynthetic pathways of GSL in plants are well known and knockout mutants lacking the gene responsible (CYP79) can be designed to create non-GSL containing plants used for experimental controls. Halkier and Gershenzon (2006) even report that future metabolic

efforts could provide the possibility to design crucifers free of certain GSLs, permitting an even deeper analysis of the effects of individual GSL on plant pathogens. Indeed crucifers always produced more than one GSL, and it is key to isolate individual GSL effects to guide crucifer selection based on levels of individual GSLs. This approach could also unravel the confusing "cocktail" effects resulting from the interaction of different GSLs produced by a single cultivar. Understanding such GSL cocktail effects is a major challenge as it could unravel unexpected causes of pathogen suppression and lead to an even higher biocontrol potential.

In the case of crucifer - legume mixtures, allelochemical cocktail effects are especially interesting to assess as legumes also have specific and different allelochemical properties. The biofumigation potential of mixtures could be more thoroughly tested against both sole crucifer and legume cover crops for the specific pathogens of interest. Mixtures containing GSL-free crucifers could also be compared especially on beneficial microbe populations and on legume growth, as GSLs produced by crucifers could impair the N services provided by the legumes. Concerning herbicide effects of crucifers, if no GSL-free cultivars are available, clues of GSL effects are still possible to achieve using experimental designs aimed to isolate allelochemical effects. For example biocidal effects of crucifers on companion legumes could be assessed in a similar way to that of Falquet et al., (2014) for weeds, by trying to isolate competition for aerial resources from the allelopathy brought by crucifers (Figure 30).

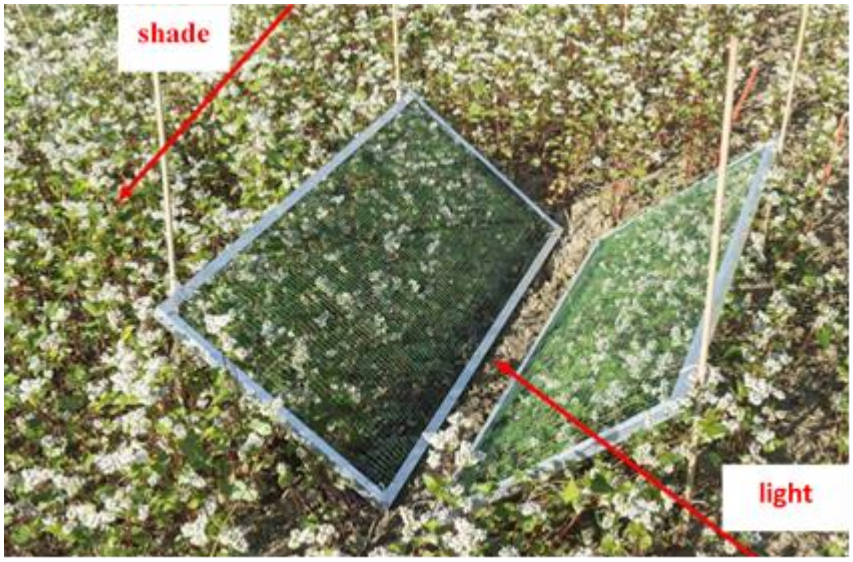

Figure 30. Experimental design to differentiate effects of light competition from other effects on pigweed suppression by buckwheat in Switzerland (© Judith Wirth).

3.2. Systems agronomy approach for better biofumigant management

Even when GSL-related effects are proven through well-chosen controls, it is always in a specific soil and climate context. Biofumigation efficacy is difficult to generalise as GSL conversion to ITCs and the fate of ITCs in soil for a targeted organism is highly dependent of pedoclimatic conditions and agronomic practices (Figure 31) (Gimsing and Kirkegaard, 2009). Thus a theoretical mechanistic "pesticide" approach linking GSL production per area and fate of GSL to predict overall toxicity of biofumigants seems complicated under field conditions due to a "Pandora's Box" phenomenon (see Figure 31). Moreover, the initial GSL production by cover crops is also dependant on these conditions and also highly variable even when using the same cultivars (Björkman et al., 2011).

A new multi-service paradigm based on a systems approach taking into account agronomic practices and other mechanisms responsible for biocontrol is needed to make better species choices and guide biofumigant management (Figure 31) (Kirkegaard and Matthiessesn 2004; Matthiessen and Kirkegaard 2006; Kirkegaard 2009). First it is important to understand when biocontrol effects occur as it can happen both during the growing period of the cover crop and after cover crop termination (e.g Motisi et al., 2009). If biocontrol mainly occurs before termination, less effort is required to optimize incorporation. Timing and termination techniques can also significantly influence efficiency of biocontrol through allelochemical fate, decomposition of organic matter and soil structure (Gimsing and Kirkegaard 2009), but to date, this has not been the focus of many field studies.

Correct assessment of biofumigation effects on pests and beneficial organisms is required to assess both services and potential disservices, and this can be challenging in the field. In that regard, novel soil ecology approaches based on new DNA monitoring of populations or inoculum levels of a wide range of both pest and beneficial organisms is a relatively new tool that is providing new insights into the effects on the soil biological systems (Canfora et al., 2016; Orgiazzi et al., 2015; Yang et al., 2014). However, practitioners must be careful to remain focussed on the most important interactions, as the amount of data emerging from these studies while interesting, can also distract attention from those interactions that drive the sustainable productivity of the system.

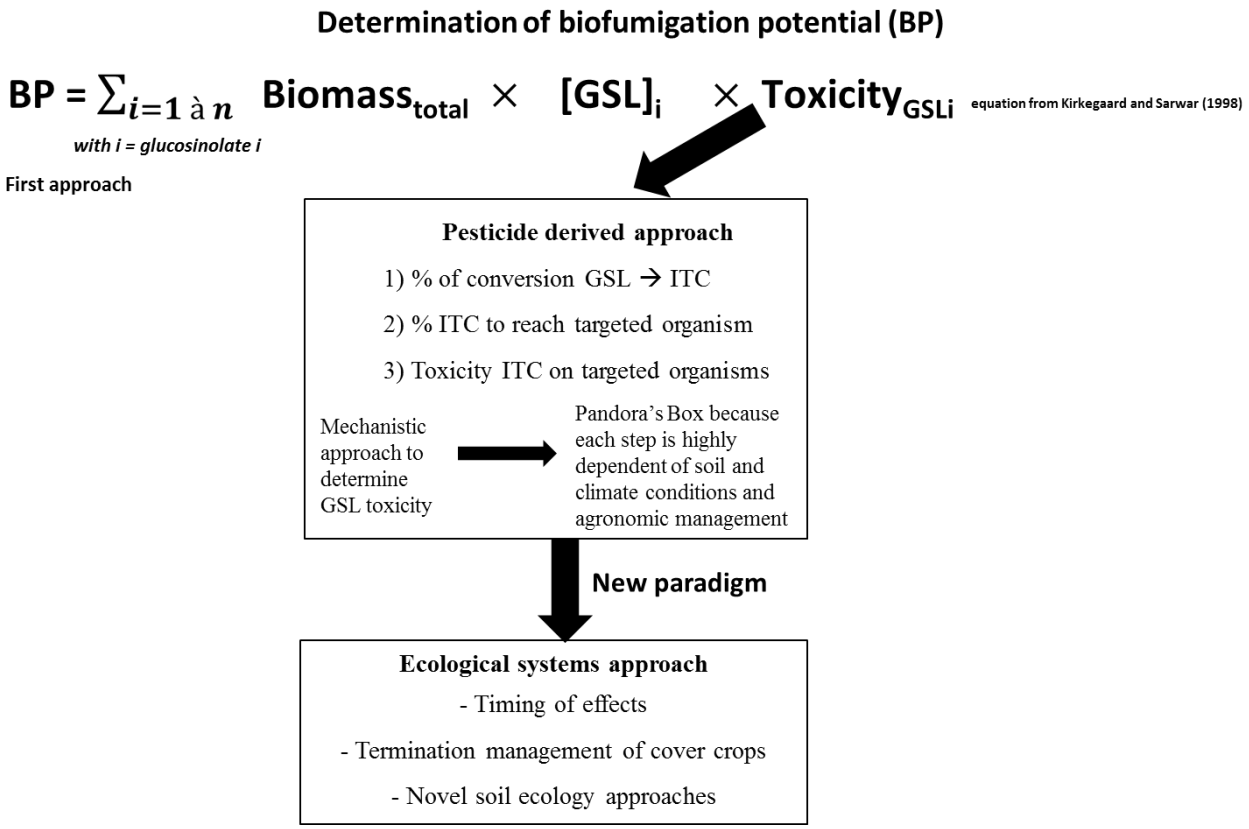

Figure 31. Representation of the pesticide based approach initially used for the biofumigation concept versus an approach designed to optimize biofumigant management for multiservice provision. (Equation from Kirkegaard and Sarwar (1998); BP = biofumigation potential for a particular target organism; Biomass total = biomass of whole cover crop; [GSL] = total GSL concentration; ToxicityGSL means toxicity of a specific glucosinolate.

3.3. Links between biotic and abiotic services of multi-service cover crops

Beyond the biotic services considered in this review, MSCC also bring many abiotic services such as improved soil properties, better nutrient cycling and greenhouse gas emission mitigation (Kaye and Quemada, 2017; Thorup-Kristensen et al., 2003). Multiservice approaches taking into account abiotic and biotic services are growing (Therond, 2017), and are key to correctly assess the overall effect of cover crops (Justes et al., 2012; 2017; Finney et al., 2017; Schipanski et al., 2014). Nevertheless in a meta-analysis, Seppelt et al. (2011) found that 50% of studies analyse individual services without considering interactions with other services. This multiservice approach has often been used at the landscape scale (Maes et al., 2012; Raudsepp-Hearne et al., 2010) but few studies at the agronomic plot or field scale assess interactions between biotic changes after cover crop use and abiotic services (Therond et al., 2017).

Two common examples of the interests of biotic services brought by cover crops leading to abiotic services are earthworm and nematode population enhancement. A well-known example of interactions between biotic and abiotic services is the role of earthworms to improve soil structure, decompose organic matter, stimulate microorganisms and contribute to the carbon and other nutrient cycling in soil (Jefferey et al. 2010). Indeed earthworms hasten the degradation and incorporation of restudies (Ortiz-Ceballos et al., 2007), improve the cohesion among and between aggregates (Kinderiene, 2006; Reeleder et al., 2006) generate better vertical porosity (Shipitalo and Le Bayon, 2004) and incorporate nutrients in aggregates (Fonte et al., 2007).

Cover cropping usually increases the diversity of beneficial nematodes communities. Nematodes are in general considered as good indicators of soil physical and chemical proprieties (DuPont et al., 2009; Porazinska et al., 1999), and the structure of the community has been linked to nitrogen (Ferris et al., 2004) and phosphorus mineralization (Takeda et al., 2009) as well as plant productivity (DuPont et al., 2009). However, clearer and convincing relationships between the populations or activities of these organisms and the functioning of the cover crop in delivering beneficial services to the system must be demonstrated to encourage adoption of such strategies.

Cover crop biomass can be considered a key indicator for both biotic and abiotic services since without sufficient growth the MSCC will not provide sufficient services and secondary metabolites may not be produced in sufficient quantity (see Figure 32). Indeed for a given cover crop species, high cover crop biomass leads to better carbon sequestration, better nutrient uptake and recycling (Thorup-Kristensen et al., 2003) as well as better soil structure through root de-compaction processes and the higher levels of organic matter added. Nevertheless high cover crop biomass can also lead to abiotic disservices, such as pre-emptive competition for water and nutrient resources compared to bare soil (Figure 32) (Thorup-Kristensen and Kirkegaard, 2016; Thorup-Kristensen et al., 2003). High cover crop biomass generally improves the suppressive mechanisms involved in pest and pathogen suppression as highlighted in Figure 32. Through an overall increase in biomass production compared to sole crops (Tribouillois et al., 2016), mixtures can improve both abiotic and biotic services to enhance better cash crop yields. Indeed the biomass produced by mixtures is enhanced due to the potential to better suppress crucifer and legume pathogens, and a better resource use efficiency (nutrients, light, water …). Over years and sites, mixtures could also be seen as a way to increase the likelihood of maintaining high levels of biomass under

variable seasons, where conditions may be less favourable for one component of the mixture, although we can find no experiments or meta-analyses to validate this hypothesis (Smith et al., 2014; S. E. Wortman et al., 2012). However in a meta-analysis dealing with harvested cash crops, Raseduzzaman and Jensen (2017) found that cereal-legume intercrops increased resilience compared to sole crops through reduced yield variability. Another interesting aspect of cover crop mixtures is their capacity to mutualize services through species characteristic diversity. Interestingly, Finney et al. (2017) concluded that multi-specific cover crop mixtures did not necessarily increase the levels of individual services compared to the best of the sole cover crops but decreased the levels of disservices generated by the sole crops. This emphasises the importance of considering all of the potential impacts of changing from sole crops to mixtures rather than focussing on a specific mechanism of interest.

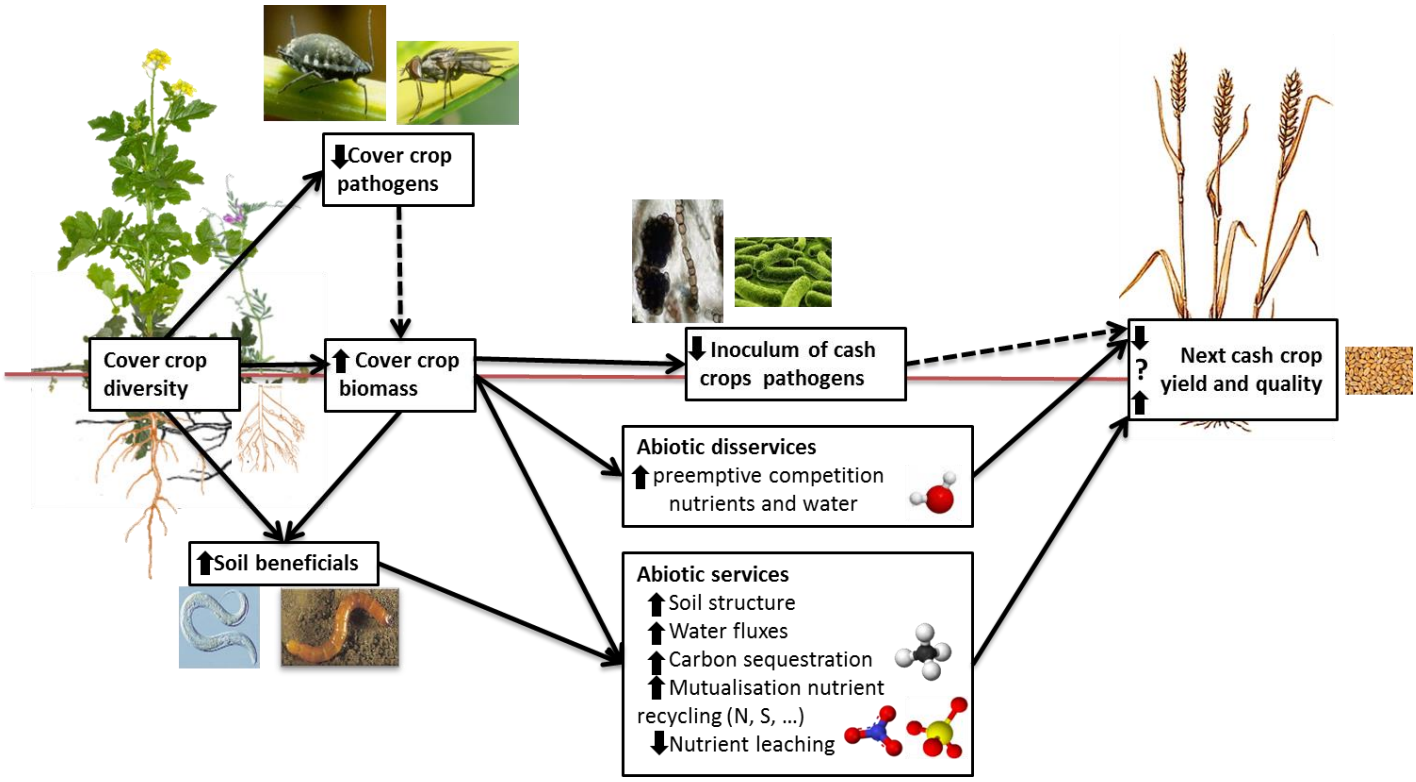

Figure 32. Biotic and abiotic services through enhanced cover crop biomass production in mixtures. Full lines represent enhanced effects while dotted lines represent decreased effects compared to sole crucifer cover crop. Black arrows represent increased (⬆) and decreased (⬇) effects in mixtures compared to sole crucifer cover crop.

3.4. The multi-service paradigm to guide cover crop selection

As cover crops can impact many aspects of the production system, a multiservice paradigm is a relevant approach. A single focus (e.g. biocontrol from specific natural compounds such as GSLs) ignores both the other services from the crucifer (organic matter input, trap crop, soil structural improvement) as well as those that may arise from a legume in the mixture (N services). A key practical question remains the selection and the assemblage of species, and also the choice of cultivars.

We propose a 3-step multi-criteria approach to assist better cover crop selection and to provide a framework to consider the merit of mixtures. The level of information required regarding some aspects of the approach also points to numerous ongoing research gaps. (Figure 33).

3.4.1. Step 1: Selection of objectives based on multi-services

Step 1 (Figure 33) considers the main services targeted and the level of compromise between services, to guide the selection of species or the proportion of species in mixtures. We assume there is no single cover crop species or approach that will suit every situation and objective.

Field characteristics such as chemical soil fertility may influence the choices made. For example on poorer soils, a high percentage of legumes in the mixture may be advised as catch crop services are less relevant, while nutrient green manure effects may be significant. In the case of more fertile soil, species with high catch crop properties could reduce nutrient losses and avoid groundwater pollution. Physical soil fertility improvement through soil de-compaction would suggest use of cover crops with strong taproots or mixtures with different rooting systems. Specific pathogen suppression may also be optimized with the use of specific crucifer or legume species Antagonisms between desired services and unwanted disservices may occur (e.g. green manure effect of legumes are incompatible with *Aphanomyces* suppression). Therefore one classification of the services according to short- and long-term effects is also important when selecting a cover species, including the profitability for farmers.

Farmers may conduct their own *in situ* experiments to address issues of interest and pertinence regarding cover crops choices according to their objectives and the desired services. Many cover crop management options may be tested prior to identifying the optimum MSCC system. The “learning curve” process of farmers must also be taken into

account on many components of cover crop management (e.g. sowing and termination methods and timing) to improve the chances of achieving the multiservices targeted (White et al., 2016). By increasing the research in these areas, advisors and producers will have access to additional resources and be better equipped to optimise cover crop techniques on their farm.

3.4.2. Step 2: Assessing and improving multi-services provided by cover crops

Selection of cover crop cultivars is significantly less advanced than for cash crops and to date has been mainly based on biomass production and nitrogen catch crop traits. Few cultivars have been specifically breed for biofumigation by maximizing GSL (or other allelochemical) production (Indian and white mustard species) (Lazzeri et al., 2004). Significant potential exists to better use the genetic variability offered by crucifer and legume cover crop species to achieve multi-services properties, but there is a great need for robust cover crop characterization of cultivars or available germplasms.

Based on our own experiments (Couëdel et al., 2018a, 2018b, 2018c), Figure 33 shows an example of multi-services to evaluate cover crops. Even on cultivars already certified and used, we report significant knowledge gaps on the capacity of cover crops to capture and recycle nutrients other than nitrogen. Nutrients such as S, Ca, Mg, K and Mg are subject to leaching and represent a major source of economic loss and pollution when lost in ground water. Other nutrients such as P are more subject to run-off and cover crops could play a key role in their recycling. In our experimental conditions, we found no major differences between cover crop species of the same family for a single abiotic service such as catch crop or green manure, but screening is needed in other conditions and for longer growing periods (fallow period >3 months) to confirm this. Despite some data on such abiotic services, data on the biocontrol potential of many cover crops (GSL production, weed control, impact on pathogen and beneficial organisms) is lacking. Potential disservices such as disease hosting, invasive characteristics of the cover crop species (through seed production) require special vigilance during the selection process.

Assessments of species mixtures performance for nitrogen-related services are available, but data are lacking on many other services. Special selection of plant traits for better complementarity of abiotic resource use and pathogen control would provide the basis to improve multiservices in mixtures (Figure 33). The ecological theory “the more diverse, the better” of cover crop mixtures must be challenged and confirmed, as bispecific mixtures

could be effective and sufficient to generate a significant improvement in multi-services provision while reducing management complexity and avoiding unwanted species competition (Smith et al., 2014).

3.4.3. Step 3: Agronomic considerations

Key technical opportunities and constraints influencing agronomic and economic cover crop performance occur mainly at sowing and termination as cover crops do not require intensive management during their growth in the fallow period between two cash crops. Sowing costs, emergence dynamics and sensitivity to termination methods are the main specific agronomic issues to take into account when choosing cover crop species (Figure 33).

Seed costs represent a significant cost of establishment (Bergtold et al., 2017), and seed costs, seeding rate and uncontrolled factors, such as seed availability can be highly variety dependant. Few studies have identified the optimal density for a targeted service (mainly a high level of biomass), yet it is the main way to reduce sowing costs. Special care has to be taken when advising seeding density as low recommendations could be risky in the case of inadequate crop emergence and could produce very heterogeneous covers. Seeding density should then be condition specific (weather, soil). Species mixtures could be seen as an interesting solution to spread the risk in the case of non-optimal crop establishment, but are generally more costly than sole crops, in particular the most common crucifers (Figure 33). Sowing of mixtures may also require species with similar seed size, as successful emergence of cover crops from a given depth is dependent on it (Tribouillois et al., 2018).

Ensuring good cover crop emergence is a major challenge and one of the main sources of variability in obtaining the desired ecosystem services in regions with hot and dry summers (Teixeira et al., 2016), with high temperatures, high evaporation and low rainfall. Therefore cover crop choice should also depend on thermic and water conditions in the seedbed since species have different sensitivity to these abiotic stresses. In a laboratory study, germination of legumes was more sensitive than crucifers to high temperatures while only radish and turnip seemed to have less water stress than other crucifer or legume species (Tribouillois et al., 2016b). Even if field data are lacking to advise on the emergence of species depending on soil and climate conditions, modelling has been used to predict emergence date of cover crops in different geographic zones depending on water availability and temperature (Constantin et al., 2015a, 2015b; Tribouillois et al., 2018). Predicting germination rate and

emergence date for different species could be especially interesting in mixtures, as it could influence the interactions and the levels of services provided.

The interaction of cover crop growth with soil type and climate, has an impact on the method of termination. Tillage and herbicides are relatively efficient termination methods whatever cover crop species is used, however these methods have many drawbacks such as environmental and economic costs (Lu et al., 2000). Other mechanical termination such as rolling or incorporation at low depth provides an alternative approach but success is highly variable between species. Mustard species are relatively easy to terminate while crucifers with strong tap root (radish and turnip) and legumes species are more difficult to kill. Frost killing is the best solution but is not always guaranteed in temperate and Mediterranean climates even during winter. Interestingly some species such as white mustard or Egyptian clover can be winter killed by frost in harsh winters reducing termination costs. Termination of cover crop mixtures is even more challenging as species can be at different growth stages, have different sensitivity to herbicides or mechanical termination methods. Selecting species with similar requirements can improve termination success.

In conclusion, despite promising techniques to successfully establish and terminate multi-services cover crops, there is little published literature on the agronomy to economically optimise management of specific cover crop species, and even less for cover crop mixtures.

3.4.3. Using the multi-service guideline

To illustrate the use of the multi-step guideline proposed in Figure 33, we provide two contrasting situations where cover crop choice is considered in different initial cropping conditions with different objectives.

In case of northern European cropping conditions, with pedoclimates and cropping systems highly sensitive to nutrient leaching (due to high rainfall during fallow period) and poor in nutrient content, the objectives of desired services (step 1) would include a strong catch crop, green manure effects and weed control. Crucifer-legume mixtures could be advised to mutualise both services of nutrient catch crop and nutrient green manure effects (Step 2). Couëdel et al. (2018c) showed good complementarity of growth for clover and vetch species with many crucifer species. Mustard - clover spp. or mustard - vetch spp. mixtures could also be a good option when targeting multi-services from cover cropping. Legume species choice could depends on climatic conditions as Egyptian clover (i.e cv. Tabor) is highly sensitive to frost while crimson clover (e.g. cv. Cegalo) is not. We showed in this review many examples

where white mustard has a strong weed suppression without generating strong disservices on beneficial organisms. Many fungal diseases can also represent a strong factor limiting cash crop production in humid climates. For example, white mustards have been showed to efficiently suppress *R. solani* in sugar beet or potato systems (Larkin and Griffin, 2007; Motisi et al., 2009) but legumes may need to be avoided as they can be host of the fungi. In that case trade-offs between disease suppression and nitrogen green manure by legumes must be considered (step 1 and 2). Step 3 shows that mustard is also easy to terminate but not clover and vetches, meaning that special care has to be taken during termination.

In case of dry fallow periods with high temperature in summer, combined with compacted soils such as in southern Europe or Australia, objectives would be to include nutrient green manure services combined with soil de-compaction. Indeed in areas with low rainfall, drainage and then nutrient leaching rarely occurs and catch crop effects of cover crops may not be important, while pre-emptive competition for water and N may be critical issues. Crucifers with strong taproots and low water use and good tolerance of stress can be advised such as radish or turnip for soil de-compaction, while a high density of legumes tolerant to temperature and water stress such as Egyptian clover could also be considered (step 2 and 3). Warmer climates can also induce higher insect pest damage on crucifer cover crops and inclusion of legume has to be considered as a repellent solution (step 1 and 2). Tall cultivars of Egyptian clovers could then be chosen as a visual and physical insect repellent to protect radishes and turnip from flies or weevils attacks for example (step 2). Despite high sensitivity of many fungal diseases to humid conditions, some are still harmful in warm and dry climates such as *G.tritici* (step 1). Canola and Indian mustard cover crops are interesting to suppress *G.tritici* and can be used in mixtures as legumes are also break crops (step 2).

In these two examples, steps 2 and 3 are interrelated and must be considered together after a first phase of objective selection. Objectives of step 1 may need to be classified by order of importance and trade-offs have to be clearly assessed, as antagonism can occur between multi-services targeted.

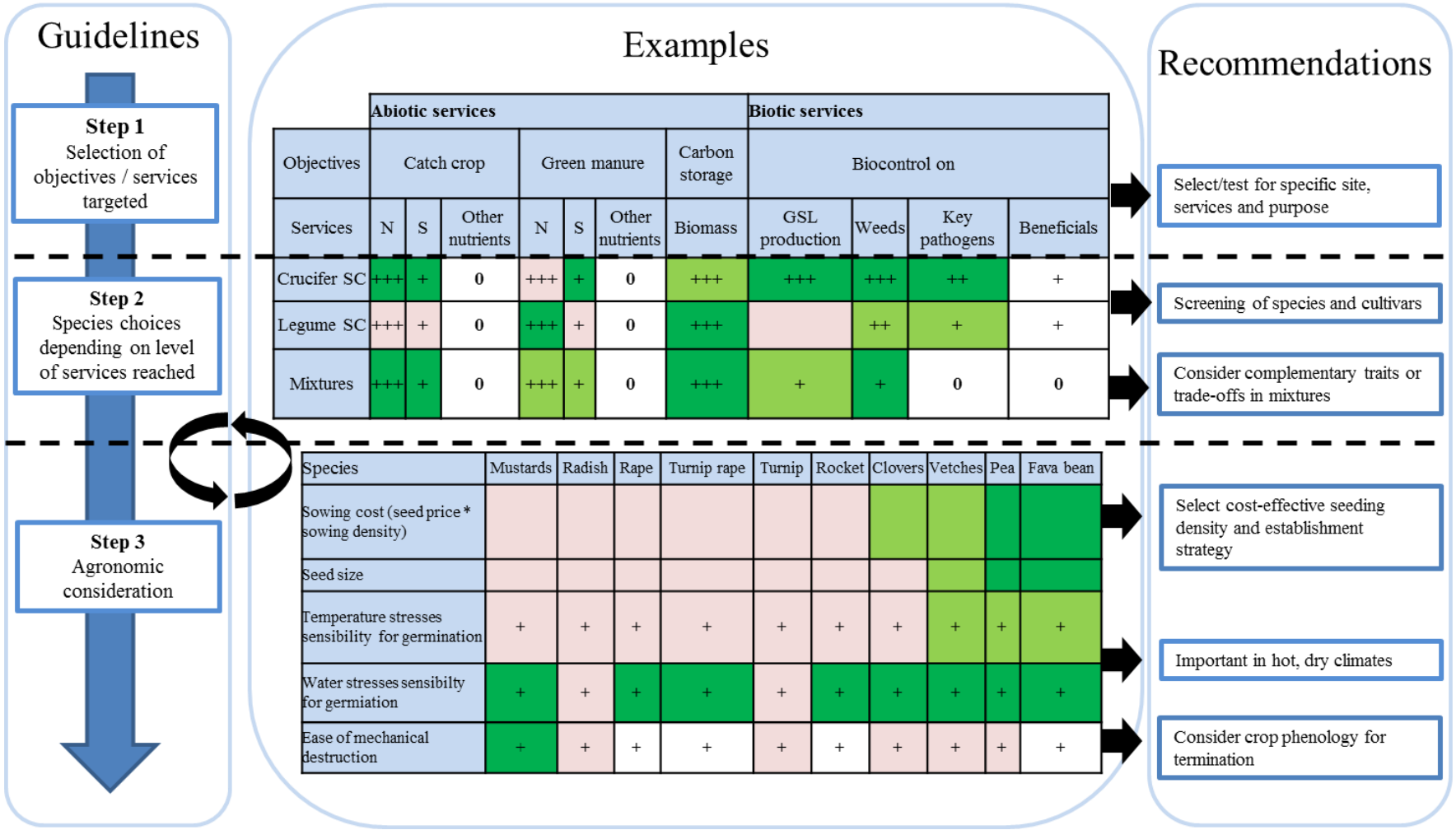

Figure 33. Three-step guideline to improve cover crop species choice. "0", "+", "++", "+++" mean respectively almost zero, low, medium and strong knowledge for each service or agronomic characteristic. Pink, light green and dark green colours mean respectively low, medium and strong level of performance for each service or agronomic characteristic. White frames mean that not enough data are available on level of performance, and may represent research opportunities.

General conclusion

This thesis demonstrates that crucifer-legume cover crop mixtures may be a promising solution to mutualise multi-services of N, S and biocontrol (based on GSL production) and increase their performances in arable cropping systems. Despite sown at half the density of sole crucifers, services of N and S catch crop were maintained at the same level in mixtures with legume, compared to sole crucifers. N green manure service reached two third compared to the sole legumes potential while S green manure service reached 85% compared to sole crucifers. As a result of a better biomass production per plant without changes in GSL types and concentration, on a ground area basis, crucifer-legume mixtures produced only 19% less GSL production compared to sole crucifer cover crops. Unchanged GSL type and concentration means that crucifers have the same potential interactions with pests and diseases. These results have been validated for a wide range of crucifer species and support the use of crucifers and legumes in mixtures to achieve multiple ecosystem services with a high level of performance.

A review of literature allow to conclude that biocontrol services of crucifers could be largely maintained in species mixtures while some of the disservices on beneficial organisms (i.e. biocidal effect) could be potentially decreased. However there is a dearth of field studies on different aspects of species mixtures for pathogen suppression, so that many of the theoretical hypotheses for the benefits of mixtures have not been demonstrated to date. It is clear that there must be special care taken in cover crop selection of species to design mixtures in order to avoid pathogen increase and incompatibility of crop growth between the species which can reduce the effectiveness of mixtures. Within the broad range of tools available in agro-ecological crop protection, multi-service cover crop mixtures cannot be seen as a stand-alone solution to manage pests and pathogens, but it is likely that adequate levels of control will require combinations with other pest management strategies.

By taking a holistic approach by using a multi-services paradigm, understanding the trade-offs between abiotic and biotic services within a true system approach is now key to assess services and disservices provided by cover crop mixtures. Selected crucifer-legume mixtures may offer promise in the quest for the sustainable intensification of agriculture and can be seen as a pillar of agroecology.

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Abstract / Résumé

Title: Provision of multiple ecosystem services by crucifer-legume cover crop mixtures

Multi-service cover crops (MSCC) grown during fallow period between two cash crops provide various ecosystem services. Among species used as MSCC, crucifers can efficiently prevent nitrate and sulphate leaching by catching residual soil mineral nitrogen (N) and sulphur (S) after the preceding cash crop (N and S catch crop services). Crucifers also have a unique capacity to suppress pathogens due to the biocidal hydrolysis products of endogenous secondary metabolites called glucosinolates (GSL). The aim of our study was to assess the provision of various ecosystem services linked to N, S cycles and biocontrol potential for a wide range of bispecific crucifer-legume mixtures in comparison to sole cover crops of legume and crucifer. We carried out experiments in 2 contrasted sites (Toulouse and Orléans regions, France) during 2 years in order to assess these services and the compatibility of various bi-specific crucifer-legume mixtures. We tested a great diversity of species, such as i) crucifers : rape, white mustard, Indian mustard, Ethiopian mustard, turnip, turnip rape, radish and rocket, and ii) legumes: Egyptian clover, crimson clover, common vetch, purple vetch, hairy vetch, pea, soya bean, faba bean, and white lupin. Our study demonstrated that crucifer-legume mixtures can provide and mutualize various ecosystem services by reaching from 2 thirds (GSL production, S and N green manure) to the same level of service (N and S catch crop) than the best sole family of species. GSL profile and concentration did not change in mixtures meaning that crucifer-pests interactions were identical. Through a literature review we also illustrated that biocontrol services of crucifers could be largely maintained in crucifer-legume mixtures for a wide range of pathogens and weeds while reducing potential disservices on beneficials and increasing N related services.

Key words: Agroecological production, mixtures, nitrogen cycle, sulphur cycle, glucosinolates, biocontrol

Titre: Analyse des performances des mélanges crucifères-légumineuses pour produire de multiples services écosystémiques en culture intermédiaire.

Les cultures intermédiaires multi-services (CIMS) implantées en interculture entre deux cultures de rente permettent de produire de nombreux services écosystémiques. Parmi les familles d'espèces utilisées comme CIMS, les crucifères réduisent efficacement la lixiviation de nitrate et de sulfate en captant l'azote (N) et le soufre (S) minéral du sol (services de piège à N et à S). Les crucifères ont aussi la capacité de contrôler les pathogènes via des composés biocides issus de l'hydrolyse de métabolites secondaires appelés glucosinolates (GSL). L'objectif de nos travaux de recherche est d'évaluer les performances en termes de services écosystémiques liés à l'azote, au soufre et au potentiel de bio-contrôle d'une grande diversité de mélanges bispécifiques de crucifères et de légumineuses en comparaison aux CIMS pures. Nous avons réalisé des expérimentations sur 2 sites contrastés (région de Toulouse et Orléans, France) et sur 2 années pour tester les performances de mélanges crucifère-légumineuse en comparaison aux espèces pures. Les espèces testées sont i) pour les crucifères : colza, moutarde blanche, moutarde brune, moutarde éthiopienne, navet, navette, radis, roquette, et ii) pour les légumineuses : trèfle Egyptien, trèfle incarnat, vesce commune, vesce pourpre, vesce velue, pois, soja, féverole et lupin blanc. Nos travaux de recherche montrent que les mélanges crucifère-légumineuse peuvent produire simultanément divers services écosystémiques avec un haut niveau d'expression, allant de 2/3 (production de GSL, engrais vert à N et S), à quasiment 100% (piège à N et S) du service produit par la famille d'espèce pure la plus performante. La concentration et les types de GSL ne changeant pas en mélanges, les interactions des crucifères avec leurs pathogènes restent identiques. Via une revue de littérature nous concluons également que le service de bio-contrôle des cultures pures de crucifères peut être maintenu en mélanges crucifère-légumineuse sur une grande diversité de pathogènes et adventices tout en réduisant les potentiels dis-services sur les auxiliaires et sur le cycle de l'azote.

Mots clefs : Production agroécologique, mélanges d'espèces, cycle de l'azote, cycle du soufre glucosinolates, bio-contrôle